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# The Effect of Inhaled FK224, a Tachykinin NK-1 and NK-2 Receptor Antagonist, on Neurokinin A-induced Bronchoconstriction in Asthmatics

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The tachykinins substance P and neurokinin A (NKA) are present in sensory airway nerves and have been implicated in the pathogenesis of asthma. FK224 is a cyclopeptide tachykinin antagonist previously shown to inhibit both tachykinin NK-1 and NK-2 receptor mediated airway responses in guinea pigs. Inhaled FK224 protected against bradykinin-induced bronchoconstriction and cough in asthmatics. In this study we examined the reproducibility of the NKA challenge and the effect of inhaled FK224 on NKA-induced bronchoconstriction in 10 patients with stable asthma. On Day 1 baseline lung function and PC<sub>20</sub> methacholine were determined. On Days 2 and 3 increasing doubling concentrations of NKA ( $3.3 \times 10^{-9}$  to  $1.0 \times 10^{-6}$  mol/ml) were administered via inhalation, with intervals of 10 min. On both days NKA caused a concentration-dependent decrease in specific airways conductance (sGaw) and FEV<sub>1</sub>. Mean  $\pm$  SEM, log PC<sub>35</sub>, sGaw NKA (mol/ml) was  $-6.61 \pm 0.10$  on Day 2 and  $-6.57 \pm 0.14$  on Day 3 (not significant [NS]). On Days 4 and 5 FK224 (4 mg) or placebo (P) was administered via metered-dose inhaler 30 min before NKA challenge in a double-blind, crossover manner. The study medication was well tolerated. FK224 had no significant effect on baseline lung function. After P and FK224, NKA caused a comparable concentration-dependent bronchoconstriction. The mean  $\pm$  SEM log PC<sub>35</sub> sGaw NKA (mol/ml) was  $-6.04 \pm 0.18$  after P and  $-6.19 \pm 0.23$  after FK224 (NS). In conclusion, inhaled FK224 had no effect on baseline lung function and offered no protection against NKA-induced bronchoconstriction in a group of mild asthmatic patients. Joos GF, Van Schoor J, Kips JC, Pauwels RA. The effect of inhaled FK224, a tachykinin NK-1 and NK-2 receptor antagonist, on neurokinin A-induced bronchoconstriction in asthmatics.

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The sensory neuropeptides substance P (SP) and neurokinin A (NKA) are present within human airway nerves (1, 2). SP and NKA contract normal human airways *in vitro* and *in vivo*, NKA being more potent than SP and asthmatics being more sensitive than normal persons (3, 4). Other potentially important airway effects of the tachykinins include mucus secretion, cough, vasodilation, increased vascular permeability, and proinflammatory effects such as the stimulation of mast cells, the stimulation of proliferation of T lymphocytes, and the chemoattraction of eosinophils and neutrophils (4-6).

SP and NKA interact with the target cells in the airways via the activation of specific tachykinin receptors, the NK-1 and NK-2 receptors. In animals, both NK-1 and NK-2 receptors have been involved in airway smooth muscle contraction, whereas in normal human airways tachykinins cause contraction by stimulation of NK-2 receptors (7-9). The tachykinin NK-1 receptor seems to be predominant in the creation of neurogenic inflammation, mucus secretion, chemotaxis, and activation of proinflammatory cells (10-12).

Lung tissue samples obtained at autopsy or thoracotomy (13), bronchoalveolar lavage fluid (14), and sputum (15) from asthmatics have all been reported to contain increased amounts of SP-immunoreactive material. Moreover, the expression of messenger RNA (mRNA) for the NK-1 receptor is enhanced in asthmatic compared with normal lung (16).

SP and NKA thus fulfill two of the three criteria to which a presumed mediator of asthma has to respond: their presence and release in the airways and their ability to mimic various features of asthma (4). Tachykinin antagonists will allow investigators to further define the role of SP and NKA in the pathogenesis of asthma and might represent a potential new class of antiasthmatic drugs.

FK224 is a recently described cyclopeptide tachykinin antagonist which inhibits NK-1 and NK-2 receptor mediated airway responses in the guinea pig (17-19). In a group of nine asthmatic subjects, FK224, 4 mg, administered by metered-dose inhaler, was shown to inhibit bradykinin-induced bronchoconstriction and cough (20). The aim of the present study was to examine the effect of inhaled FK224 on NKA-induced bronchoconstriction in asthmatics.

## METHODS

### Patients

Ten nonsmoking patients with stable mild asthma (eight males, two females, age 18 to 39 yr) participated in the study. All patients met the American Thoracic Society diagnostic criteria for asthma (21). All pa-

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TABLE 1  
CLINICAL CHARACTERISTICS OF THE ASTHMATIC SUBJECTS

Subject	Age (yr)	Sex	Baseline FEV <sub>1</sub> (ml)	Baseline FEV <sub>1</sub> (% pred)	PC <sub>20</sub> Methacholine (mg/ml)	Treatment
1	18	M	3,310	74	8.0	—
2	34	M	3,870	89	2.5	β <sub>2</sub> , St
3	22	F	2,530	79	2.0	β <sub>2</sub>
4	31	M	3,930	92	8.0	β <sub>2</sub>
5	31	M	3,810	93	8.0	β <sub>2</sub>
6	21	M	3,360	71	2.6	β <sub>2</sub> , C
7	39	M	4,950	118	7.5	β <sub>2</sub>
8	28	M	3,630	80	2.6	β <sub>2</sub>
9	22	F	3,160	87	7.7	β <sub>2</sub>
10	28	M	5,050	114	4.5	β <sub>2</sub>

Definition of abbreviations: β<sub>2</sub> = inhaled sympathomimetic; St = inhaled steroid; C = cromoglycate.

tients had positive skin prick tests to one or more common inhalation allergens. Their FEV<sub>1</sub>, expressed as mean ± SEM, was 3,760.0 ± 224.4 ml or 89.7 ± 5.0% predicted. All patients had a provocative concentration of methacholine producing a 20% fall in FEV<sub>1</sub> (PC<sub>20</sub>) ≤ 8 mg/ml. The treatment consisted of inhaled short-acting β<sub>2</sub> agonists on an "as needed" basis. One patient (Patient 2) was on inhaled steroids and one (Patient 6) on cromoglycate (Table 1). The study protocol was approved by the ethical committee of the University Hospital of Ghent. All subjects gave written informed consent.

# Lung Function Measurements

The FEV<sub>1</sub> was measured using a water-sealed spirometer (Pulmonet III; Sensormedics, Bithoven, the Netherlands). Each value represents the highest of three consecutive maneuvers. The specific airways conductance (sGaw) was calculated from airways resistance and thoracic gas volume measured with a constant volume body plethysmograph (Jaeger, Würzburg, Germany). The measurements were made with the subjects breathing quietly at rest conditions, inhaling warmed, humidified air from a separate rebreathing bag to minimize temperature and humidity artifacts. Each value represents the mean of eight consecutive maneuvers. sGaw was always measured before FEV<sub>1</sub> to prevent changes in airway caliber in response to deep inhalation.

# Bronchial Challenge Tests

The PC<sub>20</sub> for methacholine was determined by measuring the decrease in FEV<sub>1</sub> after inhalation of doubling concentrations of methacholine according to the method of Cockcroft (22).

NKA was inhaled using a protocol slightly modified from our previous work (3). After measurement of baseline FEV<sub>1</sub> and sGaw, subjects inhaled diluent (1% human serum albumin in saline). sGaw and FEV<sub>1</sub> were measured 3 and 7 min later. Provided FEV<sub>1</sub> did not change by 10% or more after inhaled diluent, increasing concentrations of NKA were inhaled (3.3 × 10<sup>-3</sup>, 10<sup>-3</sup>, 3.3 × 10<sup>-3</sup>, 1.0 × 10<sup>-2</sup>, 3.3 × 10<sup>-2</sup>, and 1.0 × 10<sup>-2</sup> moles/ml) with intervals of 10 min. sGaw and FEV<sub>1</sub> were measured 3 and 7 min after the start of the inhalation of each NKA concentration. The NKA challenge was stopped when sGaw decreased by 35% or more compared with the lowest value following inhalation of diluent.

The solutions of methacholine and NKA were nebulized with a Wiesbadener-Doppel inhalator (Wiesbadener Inhalatoren-Vertrieb, Wiesbaden, Germany) by use of an airflow of 6 L/min. The nebulized solutions were inhaled during 2 min of tidal breathing with the outlet of the nebulizer in the mouth and the nose closed by a clip. Under these conditions approximately 0.2 ml of the solution leaves the nebulizer. The patients discontinued treatment with inhaled short-acting β<sub>2</sub> agonists for at least 12 h and with sodium cromoglycate for 48 h. Inhaled steroids were continued.

# Experimental Protocol

On Visit 1 baseline lung function, PC<sub>20</sub> methacholine, and skin prick tests were done. On Visits 2 and 3 (with an interval of at least 4 d), after measurement of sGaw and FEV<sub>1</sub>, a bronchial challenge with NKA was

performed (baseline 1 and baseline 2) to determine the reproducibility of PC<sub>20</sub> sGaw NKA. The patients then entered the double-blind phase of the trial. On Visits 4 and 5 (study day 1 and study day 2, with an interval of at least 1 wk) they inhaled, in a double-blind, randomized order, 4 mg FK224 or placebo (as four puffs from a metered-dose inhaler), 30 min before NKA challenge. sGaw and FEV<sub>1</sub> were measured before, 15 min and 30 min after dosing.

# Chemicals and Drugs

NKA was obtained from Peninsula (St. Helens, UK) and was dissolved in a saline solution containing 1% human serum albumin (Behringwerke AG, Marburg, Germany). The control solution was previously shown to have no effect on airway caliber (3). The dilutions of NKA were freshly made each day and kept on ice.

Methacholine was obtained from Aldrich (Milwaukee, WI) and dissolved in phosphate-buffered saline. FK224 and placebo (metered-dose inhalers) were prepared by the sponsor Fujisawa Pharmaceuticals Co. (London, UK).

# Statistical Analysis

The baseline values for sGaw and FEV<sub>1</sub> and the log transformed values of PC<sub>20</sub> sGaw NKA were compared by the Wilcoxon matched pairs signed-ranks test. In case of a less than 35% decrease in sGaw at 1.0 × 10<sup>-6</sup> moles/ml, the value of PC<sub>20</sub> sGaw NKA was taken as 3.3 × 10<sup>-6</sup> moles/ml. Treatment with FK224 was compared with placebo by means of a three-way analysis of variance (ANOVA). The different concentration points were compared two by two by the Wilcoxon's matched pairs signed-ranks tests (Primer, Version 1.0, McGraw-Hill 1988; Systat Version 5.01, Systat Inc., 1991).

The results of the bronchial challenge with NKA are expressed as the percentage changes in sGaw and FEV<sub>1</sub>. The data are reported as the mean ± standard error of the mean (SEM). A p value < 0.05 was regarded as significant.

# RESULTS

Neurokinin A caused a concentration-dependent bronchoconstriction in the 10 asthmatic patients studied. The effect of NKA was reproducible from baseline 1 to baseline 2 (Table 2 and Figure 1).

On both treatment days, baseline sGaw and FEV<sub>1</sub> were similar (mean ± SEM sGaw on the placebo day: 0.097 ± 0.014 versus FK224: 0.109 ± 0.015 cm H<sub>2</sub>O/s; mean ± SEM FEV<sub>1</sub> on the placebo day: 3,658 ± 249 versus FK224: 3,670 ± 213 ml; Mann-Whitney U test: not significant for both sGaw and FEV<sub>1</sub>). The inhalation of FK224 caused no significant changes in baseline lung function: the percentage change in sGaw after placebo was -5.2 ± 9.6% at 15 min and +2.5 ± 7.5% at 30 min; the percentage change in sGaw after FK224 was -1.1 ± 6.8% at 15 min and +19.3 ± 6.2% at 30 min; the percentage change in FEV<sub>1</sub> after placebo was -2.3 ± 1.4% at 15 min and -1.4 ± 1.0% at 30 min; the percentage change in FEV<sub>1</sub> after FK224 was +1.6 ± 1.9% at 15 min and 3.0 ± 1.1% at 30 min.

Thirty minutes after pretreatment with FK224 (4 mg) or placebo, similar concentration-dependent changes in sGaw and FEV<sub>1</sub> were observed after inhalation of NKA (Figure 2). The logarithm of the PC<sub>20</sub> sGaw NKA was similar on both study days, for all subjects studied (Table 2). The mean maximal percent decrease sGaw after placebo was 38.4 (SEM 6.5) and after FK224 44.4 (SEM 8.3) (Mann-Whitney U test: not significant). The mean maximal percent decrease FEV<sub>1</sub> after placebo was 8.7 (SE 2.3) and after FK224 11.9 (SEM 3.7) (Mann-Whitney U test: not significant).

# DISCUSSION

In this study the cyclopeptide NK-1 and NK-2 tachykinin receptor antagonist FK224, given by metered-dose inhaler at a dose of 4 mg, did not prevent the bronchoconstriction induced by in-

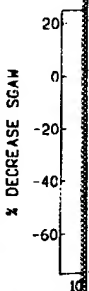


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TABLE 2  
LOGARITHMICALLY TRANSFORMED INDIVIDUAL VALUES  
FOR THE CONCENTRATION OF NKA CAUSING A 35% DECREASE  
IN sGaw (logPC<sub>35</sub> sGaw NKA) (MOL/ML) IN  
THE 10 ASTHMATIC SUBJECTS STUDIED

Subject	Baseline 1	Baseline 2	Placebo	FK224
1	-6.39	-6.12	-6.26	-6.02
2	-6.44	-6.00	-6.18	-6.10
3	-6.16	-6.67	-6.27	-6.38
4	-6.14	-6.25	-5.48	-6.13
5	-6.83	-6.42	-5.48	-5.48
6	-6.85	-7.24	-7.16	-7.69
7	-7.04	-6.62	-5.48	-6.00
8	-6.71	-7.18	-6.46	-7.20
9	-6.79	-6.15	-5.48	-5.48
10	-6.78	-7.03	-6.10	-5.48
Mean $\pm$ SEM	-6.61 $\pm$ 0.10	-6.57 $\pm$ 0.14	-6.03 $\pm$ 0.18	-6.10 $\pm$ 0.23

haled neurokinin A in patients with asthma. Furthermore, no significant changes in baseline airway caliber were observed. Various elements could account for this lack of effect.

The bronchoconstriction occurring after inhalation of neurokinin A was found to be reproducible. Although ANOVA revealed significant differences between the changes in sGaw on baseline 1 and 2, a point-by-point analysis of the concentration-response curves by the Wilcoxon test revealed no significant differences (Figure 1). Moreover the PC<sub>35</sub> sGaw for neurokinin A and the changes in FEV<sub>1</sub> were very similar on both baseline days.

The PC<sub>35</sub> sGaw neurokinin A values on the baseline days differed by 0.5 log from the PC<sub>35</sub> sGaw neurokinin A values observed on the two study days, suggesting that on the study days the patients were less responsive to neurokinin A. However, these differences were not significant. In addition, we are not aware of any data suggesting a diminished bronchial reaction to neurokinin A over time. It has also to be borne in mind that inhaling medication (albeit placebo) can influence a subsequent bronchial response.

Three-way ANOVA to compare the effect of FK224 and placebo on neurokinin A-induced decreases in sGaw demonstrated a significant difference between placebo and FK224. However, the direction of the difference was opposite to what one would expect from a receptor antagonist. Moreover, ANOVA did not reveal a difference for the changes in FEV<sub>1</sub> on both study days.

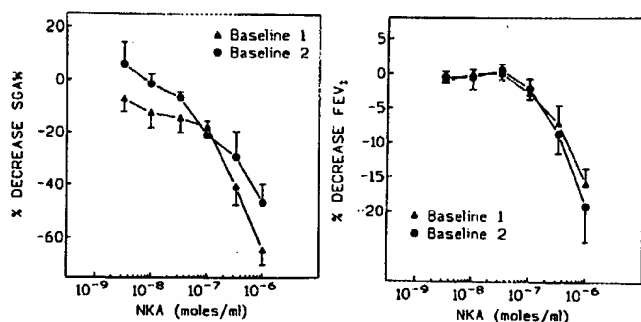


Figure 1. Mean percentage change ( $\pm$  SEM) in sGaw and FEV<sub>1</sub> 3 min after inhalation of increasing concentrations of neurokinin A (NKA) in the asthmatic subjects on Visits 2 and 3 (respectively baseline 1 and baseline 2) ( $n = 10$ ). ANOVA: baseline 1 versus baseline 2,  $p = 0.007$  for changes in sGaw and  $p = 0.68$  for changes in FEV<sub>1</sub>. Wilcoxon matched-pairs tests for comparison of the changes in sGaw at baseline 1 versus baseline 2 at the different concentration points: no significant differences.

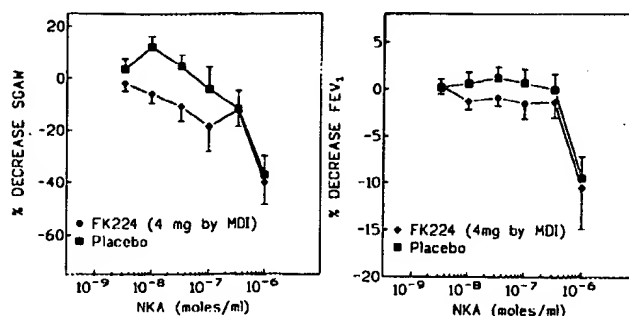


Figure 2. Mean percentage change ( $\pm$  SEM) in sGaw and FEV<sub>1</sub> 3 min after inhalation of increasing concentrations of neurokinin A (NKA) in the asthmatic subjects on the two study days ( $n = 10$ ). ANOVA: FK224 versus placebo  $p = 0.003$  for changes in sGaw,  $p = 0.159$  for changes in FEV<sub>1</sub>; Wilcoxon matched-pairs test  $p = 0.009$  for change in sGaw at NKA  $10^{-8}$  mol/ml,  $p = 0.047$  for NKA  $3.0 \times 10^{-8}$  mol/ml (all other pairs being not significantly different).

FK224 was found to exert antagonism for both the NK-1 and the NK-2 tachykinin receptor in various animal models (17–19, 23). The reported affinity of the antagonist for the receptor (pA<sub>2</sub>) values were, however, quite low (e.g., 6.88 for substance P-induced contraction of guinea pig ileum and 7.50 for neurokinin A-induced contraction of rat vas deferens). One could therefore speculate that the given dose of FK224 was insufficient to effectively block NK-1 and NK-2 receptors in the airways. However, FK224 was previously shown to offer protection against bradykinin-induced bronchoconstriction in asthmatics (20). The experimental setting used in this study by Ichinose and coworkers was very similar to our protocol. The same dose (4 mg given by metered-dose inhaler) was applied and the time schedule of administration of the drug and the duration of the provocation test were close to ours (20). It is therefore unlikely that the lack of effect observed in our study is due to rapid inactivation of FK224. Another explanation for our negative results could be the use of a less potent or older batch of the metered-dose inhaler of FK224. However, the activity of the compound was tested by Fujisawa, and the medication was administered before the expiry date.

FK224 has previously been shown to interact with the effect of tachykinins on isolated human airways. In a preliminary report, Yamaguchi and coworkers demonstrated that high concentrations ( $10^{-5}$  M) of FK224 significantly inhibited neurokinin A and capsaicin-induced contractions of isolated human bronchi (24). In a recent paper, Walsh and coworkers reported that FK224 inhibited the [<sup>125</sup>I]-Bolton Hunter labeled SP binding in human bronchial vessels. This effect occurred with low affinity when compared with substance P and the specific NK<sub>1</sub> receptor antagonist FK888 (K<sub>i</sub> 434 nM for FK224 versus 0.8 nM for SP and 0.8 nM for FK888) (25).

Based on studies in guinea pigs it was suggested that bradykinin induces bronchoconstriction by release of tachykinins from sensory nerves (26, 27). The protective effect of FK224 against bradykinin challenge in asthmatics observed by Ichinose and coworkers suggested that this mechanism was also present in the asthmatic airway (20). The results of our study make this explanation very unlikely. Moreover, it was recently shown that the contractile effect of bradykinin in normal, small human airways was not affected by pretreatment with specific and potent tachykinin receptor antagonists (28).

In studies in guinea pigs FK224 was found to be rather specific for NK-1 and NK-2 receptors and to offer no protection

against the contractile effect of acetylcholine and histamine or the airway edema caused by histamine (18, 29). Although FK224 had no effect on the binding of bradykinin to guinea pig lung membrane (Fujisawa, FK224 Investigator Brochure), it remains however possible that the protective effect of FK224 on bradykinin-induced bronchoconstriction in asthmatics was merely due to antagonism at the bradykinin receptor.

In conclusion, our study suggests that FK224 is not a good tool to study the role of tachykinins in human airway diseases. Moreover, the previously observed protective effect of FK224 against bradykinin challenge cannot be explained by antagonism for NKA.

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# Role for Neurokinin-2 Receptor in Interleukin-5-induced Airway Hyperresponsiveness but not Eosinophilia in Guinea Pigs

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In the guinea pig, interleukin-5 (IL-5) has been shown to induce airway hyperresponsiveness as well as eosinophilia, which are important symptoms in asthma. IL-5 seems to be a critical cytokine since it selectively affects eosinophil functions. The mechanism of action by which IL-5 leads to airway hyperresponsiveness may be important for our understanding of the pathogenesis of asthma. Neurogenic inflammation, which is mediated by nonadrenergic noncholinergic nerves (NANC), may play a role in the IL-5-induced effects in guinea pig airways. In this study, the role of neuropeptides in the IL-5-induced airway hyperresponsiveness and eosinophilia in the guinea pig was examined using selective neurokinin receptor antagonists. Intra-airway application of IL-5 (1  $\mu$ g, twice) induces a selective eosinophil migration (control:  $12 [8-22] \times 10^5$  cells and IL-5:  $90 [67-187] \times 10^5$  cells,  $p < 0.05$ ) and activation (control:  $6.3 \pm 0.9$  ng eosinophil peroxidase [EPO]/ml bronchoalveolar lavage [BAL] fluid and IL-5:  $29.3 \pm 4.9$  ng EPO/ml BAL fluid,  $p < 0.05$ ) and a pronounced airway hyperresponsiveness *in vivo*. The maximal responses to histamine are increased by  $160 \pm 16\%$  ( $p < 0.05$ ) after IL-5. Treatment of guinea pigs with either the nonselective neurokinin (NK)-receptor antagonist, FK224, or the selective NK2-receptor antagonist, SR48968, results in a complete inhibition of the *in vivo* hyperresponsiveness found after application of IL-5. Vice versa, intra-airway administration of substance P (10  $\mu$ g, twice) results in an airway hyperresponsiveness (increased maximal response after substance P:  $166 \pm 15\%$  [ $p < 0.05$ ]) without inducing migration or activation of eosinophils. All examined NK-receptor antagonists do not influence the IL-5-induced eosinophil accumulation. In addition, no effect of the NK-receptor antagonists is observed on the IL-5-induced eosinophil activation, as determined by BAL fluid EPO levels. The release of NK2-receptor active tachykinins plays an important role in the development of IL-5-induced airway hyperresponsiveness. This feature appears to be a step following eosinophil infiltration and activation since there are no effects on eosinophil function by pretreatment of the used NK-receptor antagonists. Kraneveld AD, Nijkamp FP, Van Oosterhout AJM. Role for neurokinin-2 receptor in interleukin-5-induced airway hyperresponsiveness but not eosinophilia in guinea pigs.

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Infiltration into the airways of inflammatory cells, particularly T lymphocytes and eosinophilic granulocytes, and pulmonary hyperresponsiveness to bronchoconstrictor mediators are prominent features of the pathogenesis of asthma (1). However, the exact mechanistic relationship between airway hyperresponsiveness and leukocyte infiltration is still unknown. Recent studies suggest a role for T helper 2 lymphocyte-derived cytokines such as interleukin-5 (IL-5) in the development of airway eosinophilia and hyperresponsiveness (2, 3). We and oth-

ers have demonstrated that antibodies to IL-5 inhibited the airway hyperreactivity and bronchoalveolar eosinophilia in a guinea pig model for allergic asthma (4, 5). IL-5 is reported to be a selective activator of eosinophils, which promotes growth, differentiation, proliferation and survival of eosinophils (6, 7). In addition, IL-5 is an eosinophilic chemoattractant and primes eosinophils for enhanced chemotaxis and leukotriene production in response to several inflammatory mediators (8-10). Therefore, this cytokine may be responsible for the selective recruitment and activation of eosinophils at sites of allergic reactions.

Indeed, administration of IL-5 has been demonstrated to induce airway eosinophilia and hyperreactivity in guinea pigs and mice (4, 11-13). Very recently, in the guinea pig we have shown that for the development of IL-5-induced airway hyperresponsiveness *in vivo*, the very late activation antigen-4 (VLA-4) dependent infiltration of eosinophils into the bronchial tissue is essential (14). VLA-4 is an important and selective adhesion molecule in the infiltration of eosinophils, but

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not neutrophils, from the vasculature to the airway tissue. These data underline the putative role of eosinophils in the development of pulmonary hyperresponsiveness.

In the airways it has been well recognized that nonadrenergic noncholinergic nerves (NANC) form part of the local nervous system. Neurogenic inflammation, which is mediated by NANC nerves, may play a role in asthma (15). It is now known that NANC neuropeptides, such as the tachykinins, substance P, or neurokinin A, have potent inflammatory effects and can affect airway function in a way which resembles features found in the pathogenesis of asthma. Several studies have reported that exposure of guinea pigs to an aerosol of either capsaicin, a substance releasing endogenous NANC neuropeptides, or substance P elicited airway hyperresponsiveness to bronchoconstrictor agents (16–18). In addition, in a guinea pig model for allergic asthma, depletion of NANC neuropeptides by chronic capsaicin pretreatment, resulted in a profound inhibition of antigen-induced airway hyperresponsiveness (19, 20). However, the antigen-induced eosinophilia was still evident (20). In addition, van Oosterhout and colleagues have shown in the guinea pig that after *in vivo* administration of either IL-5 or substance P, tracheal responses to histamine were significantly increased (13). Application of IL-5, but not of substance P, induced a significant increase in the number of eosinophils as well as a rise in eosinophil peroxidase activity (a marker for eosinophil activation) in bronchoalveolar lavage fluid of guinea pigs (13). These results suggest that IL-5 is important in the recruitment and activation of eosinophils, whereas NANC neuropeptides seem to be involved in the process by which eosinophils induce hyperreactivity.

To investigate the role of tachykinins in the development of changes in airway function, the effect of several neurokinin receptor antagonists was studied on IL-5-induced airway hyperresponsiveness and eosinophilia in the guinea pig.

## METHODS

### Animals

The animals used in this study were specified pathogen-free male Dunkin Hartley guinea pigs weighing 390–550 g (Harlan Porcellus, Blackthorn, Bicester, Oxon, UK). Water and commercial chow were allowed *ad libitum*. The guinea pigs were free of respiratory infections as assessed by the health monitoring quality control report by Harlan Porcellus. The experiments were approved by the Animal Care Committee of the Utrecht University (Utrecht, The Netherlands).

### Intra-airway Administration of IL-5 or Substance P

Guinea pigs received either 1  $\mu$ g IL-5, 10  $\mu$ g substance P, or vehicle consisting of saline with 0.1% bovine serum albumin (BSA) in a volume of 300  $\mu$ l intranasally under short-lasting anesthesia (Ketalar<sup>®</sup>, 40 mg/kg intramuscularly, Parke-Davis, Hoofddorp, The Netherlands; and Rompun<sup>®</sup>, 5 mg/kg subcutaneously, Bayer BV, Mijdrecht, The Netherlands) twice on one day (9:00 A.M. and 4:00 P.M.).

### Neurokinin Receptor Antagonist Treatment

The nonselective neurokinin receptor antagonist, FK224, and the neurokinin-1 receptor antagonist, FK888, were dissolved in 0.1% ethanol and injected intravascularly at a dose of 1  $\mu$ mol/kg and 2  $\mu$ mol/kg body weight, respectively, 30 min before each IL-5 or vehicle administration. The selective neurokinin-2 receptor antagonist, SR 48698, was dissolved in saline and injected intraperitoneally at a dose of 1 mg/kg body weight, 1 h before each IL-5 or vehicle administration. The dose and route of administration of neurokinin receptor antagonists were selected based on results showing the effects of FK224, FK888, or SR 48698 on tachykinin- or antigen-induced bronchoconstriction in guinea pigs (21–23). In addition, in a pilot experiment it was demonstrated that pretreatment with FK888 (1  $\mu$ mol/kg body weight) resulted in a  $61.9 \pm 0.3\%$  (mean  $\pm$  STD,  $n = 2$ ) of substance P-induced decrease in blood pressure.

Hirayama and colleagues (23) examined the non-specific actions of FK224 and FK888 on PAF-induced plasma exudation and bronchoconstriction in capsaicinized guinea pigs. They have demonstrated a complete lack of inhibitory effect of FK224 and FK888. Moreover, in the guinea pig, it is shown that SR48698 did not affect acetylcholine-induced bronchoconstriction or histamine-induced effects on airway resistance and blood pressure (21, 37). Thus it can be concluded that there are no nonspecific actions for the antagonists used in this study.

### Airway Reactivity *in vivo*

Twenty-four hours after the first administration of IL-5 or vehicle, the guinea pigs were prepared for measurement of lung resistance ( $R_L$ ). The animals were anesthetized with urethane (2.8 g/kg intraperitoneally) and were able to breathe spontaneously. To avoid an anesthesia-induced fall in body temperature the animals were placed in a heated chamber at approximately 30° C. To determine airflow (V) and tidal volume (V<sub>T</sub>) the trachea was cannulated and connected to a Gould Godart pneumotachograph (Gould Godart, Bunnik, The Netherlands) with a Fleisch flow head (No. 000; Meijnhart, Bunnik, The Netherlands). A Validyne MP45-24 pressure transducer (Validyne Engineering Corp., Northridge, CA) measured the pressure difference between the tracheal cannula and a cannula filled with saline inserted in the esophagus, which presented the transpulmonary pressure (TPP).  $R_L$  was determined breath by breath by the method of Amdur and Mead (24) using a computerized respiratory analyzer. Dividing  $\Delta$ TPP by  $\Delta$ V at isovolume points (50%) yielded the  $R_L$ . A small polyethylene catheter used for intravenous administration of histamine was placed in the right jugular vein. A histamine dose-response (2 to 20  $\mu$ g/kg) curve was made. Injections were given at intervals of at least 5 min in which  $R_L$  returned to baseline. Responses are presented as increases in  $R_L$  above baseline.

### Bronchoalveolar Lavage

Bronchoalveolar lavages (BAL) were performed in all guinea pigs used. After measurement of lung resistance, the animals received a lethal dose of pentobarbital sodium (300 mg/kg intraperitoneally). Via the tracheal cannula, the lungs were filled *in situ* with 5 to 10 ml NaCl-EDTA-buffer (0.15 M NaCl, 2.6 mM EDTA) using a syringe. Fluid was withdrawn from the lungs and collected in a plastic tube on ice. The first lavage (approximately 5 ml) recovered from each animal was kept separate, and the lavages thereafter were pooled until 50 ml fluid was obtained. The cells were sedimented by centrifugation at 400 g for 10 min at 4° C. The supernatant of the first lavage (cell-free BAL fluid) was stored at –70° C prior to analysis of BAL fluid eosinophil peroxidase activity. The two cell pellets from the lavages were resuspended and pooled. The cells were washed twice with NaCl-EDTA-buffer. A sample of the cells were stained with Turk solution and counted. All cell preparations were analyzed morphologically after centrifugation on microscopic slides. Air-dried preparations were fixed and stained with Diff-Quik (Merz & Dade A.G., Dudingen, Switzerland). Differential counts were made under oil immersion microscopy.

### BAL Fluid Eosinophil Peroxidase Activity

The eosinophil peroxidase (EPO) activity in the supernatant of the first lavage (cell-free BAL fluid) was measured according to the method of Strath and coworkers (25), which is based on the oxidation of O-phenylenediamine (OPD) by EPO in the presence of hydrogen peroxide ( $H_2O_2$ ). The substrate solution consisted of 10 mM OPD in 0.05 M Tris-buffer (pH = 8) and 4 mM  $H_2O_2$  (BDH, Poole, UK). Substrate solution (100  $\mu$ l) was added to BAL cell supernatant samples (50  $\mu$ l) in a 96-wells microplate and incubated at room temperature for 30 min before stopping the reaction by addition of 50  $\mu$ l of 4 M sulfuric acid. The absorbance was then measured at 492 nm using a Titertek Multiskan (Flow Labs., Irvine, UK). Duplicate incubations were carried out in the absence and presence of the EPO inhibitor 3-amino-1,2,4-triazole (AMT, 2 mmol/l). Blanks were cell free BAL fluid samples (50  $\mu$ l) incubated with Tris-HCl buffer. Serial dilutions of horseradish peroxidase (200 ng/ml) were used to quantitate the amount of peroxidase in the samples. Results are expressed as ng/ml peroxidase activity and were corrected for the activity of other peroxidases, which were not inhibitable by AMT.



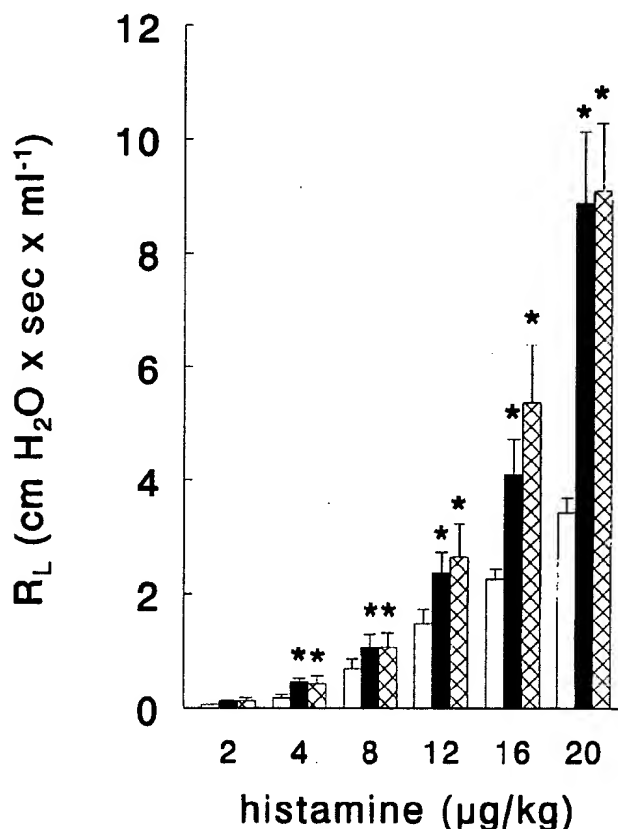


Figure 1. Increase in lung resistance ( $R_L$ ) after intravenous administration of histamine to guinea pigs one day after two intranasal treatments with 1  $\mu$ g IL-5 ( $n = 5$ , closed bars), 10  $\mu$ g substance P ( $n = 5$ , hatched bars) or vehicle ( $n = 5$ , open bars). \* $p < 0.05$  as compared with vehicle treated animals, ANOVA, Bonferonni test.

## Materials

Substance P, *O*-phenylenediamine, 3-amino-1,2,4-triazole, horseradish peroxidase, and bovine serum albumin grade V were obtained from Sigma Chemical Company (St. Louis, MO). The human recombinant IL-5 was purified by Dr. A. E. Proudfoot (Glaxo Institute for Molecular Biology, Geneva, [26]) and kindly donated by Dr. D. Fatah (Glaxo, Greenford, UK). The dipeptide neurokinin-1 receptor antagonist FK888, N2[(4R)-4-hydroxy-1-(1-methyl-1H-indol-3-yl)carbonyl-L-propyl]-N-methyl-N-phenylmethyl-3-(2-naphthyl)-L-alaninamide, and the peptidergic neurokinin1/2 receptor antagonist, FK224, N-(N<sub>2</sub>-(N-(N-(2,3-didehydro-N-methyl-N-(N-(3-(2-pentylphenyl)-propionyl)-L-threonyl)tyrosyl-L-leucyl)-D-phenylalanyl)-L-allo-threonyl)-L-asparaginyl-L-serine- $\gamma$ -lactone, were gifts from Fujisawa Pharmaceuticals Co. Ltd. (Osaka, Japan). SR 48968, (S)-N-methyl-N(4-acetyl-amino-4-phenylpiperidino-2-(3,4-dichloophenyl)butyl)benzamide, was kindly donated by Sanofi Recherche (Montpellier, France).

## Data Analysis

The cellular accumulation in BAL fluid was analyzed by using a distribution-free Kruskal-Wallis one-way analysis of variance test. The cell data are expressed as medians (minimum-maximum).

The EPO activity data and airway reactivity data are expressed as mean  $\pm$  SEM. These data were initially analyzed using Bartlett's test for homogeneity of variances. For the raw EPO activity data the Bartlett's test indicated no heterogeneity of variance. Analysis of the raw airway resistance data with the Bartlett's test demonstrated a significant difference between group variances. However, the group variances of log-transformed data were homogenous. All subsequent analyses were done on raw EPO activity data and log-transformed airway resistance data using the parametric analysis of variance (ANOVA). Differences between groups were tested with a Bonferonni post-hoc test. A  $p$  value  $< 0.05$  was considered to reflect a statistically significant difference. All analyses were performed by using SYSTAT (version 5.03, Wilkinson, Leland, SYSTAT: the system for statistics, Evanston, IL., Systat Inc., 1990).

## RESULTS

### IL-5-induced Changes in Guinea Pig Airways

One day after intra-airway application of IL-5 (twice: 1  $\mu$ g) bronchial hyperresponsiveness to histamine *in vivo* in the guinea pig was observed (Figure 1). In IL-5-treated animals a dose of 20  $\mu$ g/kg histamine resulted in an increase in lung resistance of  $160 \pm 16\%$  when compared with vehicle treatment ( $p < 0.05$ ,  $n = 5$ , ANOVA). The change in bronchial reactivity was associated with a marked accumulation of eosinophils into the airways (Table 1). Furthermore, an enhanced EPO activity in cell free BAL fluid (control:  $6.3 \pm 0.9$  ng/ml and IL-5:  $29.3 \pm 4.9$  ng/ml,  $p < 0.05$ ,  $n = 5$ , ANOVA) was observed after intra-airway administration of IL-5 indicating that the eosinophils were activated.

### Substance P-induced Changes in Guinea Pig Airways

One day after intra-airway administration of substance P (twice: 10  $\mu$ g) the increases in airway resistance to increasing doses of histamine were significantly enhanced (Figure 1). The maximal increase in lung resistance to a dose of 20  $\mu$ g/kg histamine potentiated with  $166 \pm 15\%$  in substance P-treated animals when compared with vehicle-treated animals ( $p < 0.05$ ,  $n = 5$ , ANOVA). The substance P-induced airway hyperresponsiveness was not accompanied by a rise in number of eosinophils in the BAL fluid nor by changes in EPO activity in cell free BAL fluid (Table 1 and Figure 2).

### Effects of Neurokinin Receptor Antagonists on IL-5-induced Changes in Guinea Pig Airways

The effects of the non-selective neurokinin (NK) receptor antagonist, FK224, the neurokinin-1 (NK1) receptor antagonist, FK888, and the neurokinin-2 (NK2) receptor antagonist, SR-48968, were studied on the IL-5-induced bronchial hyperresponsiveness, eosinophil accumulation and activation in the guinea pig airways. Pretreatment of the guinea pigs with either

TABLE 1  
NUMBER OF DIFFERENT CELL TYPES ( $\times 10^5$ ) IN BRONCHOALVEOLAR LAVAGE FLUID ONE DAY AFTER INTRA-AIRWAY ADMINISTRATION OF IL-5, SUBSTANCE P OR VEHICLE IN GUINEA PIGS

Treatment	n	Eosinophilic Granulocytes	Neutrophilic Granulocytes	Mononuclear Cells
Vehicle	5	12 (8-22)	54 (44-83)	130 (96-187)
IL-5	5	90 (67-187)*	75 (69-109)	134 (97-180)
Substance P	5	17 (12-26)	34 (22-84)	110 (86-151)

The results are expressed as median (minimum - maximum).

\*  $p < 0.05$  as compared with vehicle-treated animals, distribution-free Kruskal-Wallis one-way analysis of variance test.

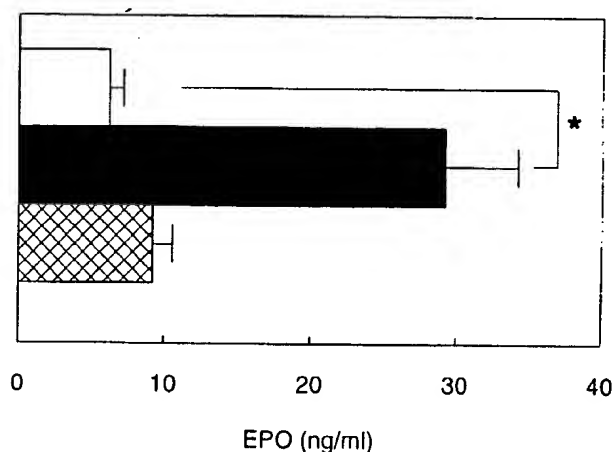


Figure 2. Eosinophil peroxidase (EPO) activity (ng/ml) in cell free BAL fluid obtained from guinea pigs one day after two intranasal treatments with vehicle ( $n = 5$ , open bars), or  $1 \mu\text{g}$  IL-5 ( $n = 5$ , closed bars) or  $10 \mu\text{g}$  substance P ( $n = 5$ , hatched bars). \* $p < 0.05$  as compared with vehicle treated animals, ANOVA, Bonferonni test.

FK224 (NK receptor antagonist) or SR 48968 (NK2 receptor antagonist) resulted in a complete inhibition of the *in vivo* bronchial hyperresponsiveness found 24 h after intra-airway administration of IL-5 (Figures 3B and 4). In contrast, the NK1 receptor antagonist, FK888, did not influence the IL-5-induced responses on airway resistance to histamine in the guinea pig (Figure 3C). The three examined NK receptor antagonists did not influence airway resistance to histamine in control animals. There were no statistically significant differences between all control groups.

All examined NK receptor antagonists did not affect the IL-5-induced eosinophil accumulation into the BAL fluid (Figure 5). After pretreatment with FK224, FK888, or SR 48968, intra-airway administration of IL-5 resulted in a com-

parable and significant increase in the numbers of eosinophils in the BAL fluid when compared with vehicle pretreatment. In addition, no effects of the NK receptor antagonists were observed on the IL-5-induced increase in EPO activity in the cell free BAL fluid of the guinea pig airways (Figure 6).

## DISCUSSION

In this study, we have demonstrated that the neurokinin-2 receptor plays an important role in the development of IL-5-induced hyperresponsiveness but not in the eosinophil infiltration into the airways of guinea pigs.

The T helper 2 lymphocyte-derived cytokine IL-5 has been shown to induce bronchial hyperresponsiveness as well as airway eosinophilia, which are important symptoms in asthma (4, 11–14, 27). In asthmatic patients, IL-5 is produced locally in the airways and this phenomenon is related to the number of eosinophils in the airways (3, 28). Furthermore, in allergic asthma mRNA expression of T helper 2 cytokines, in particular IL-5, is associated with clinical measures of asthma severity, i.e., airflow restriction and hyperresponsiveness to bronchoconstrictor agents (28, 29). Although IL-5 probably acts in concert with other cytokines, IL-5 emerges as a critical cytokine in asthma. To understand the importance of IL-5 in the pathogenesis of asthma, it is very relevant to try to elucidate the possible mechanism of action of IL-5.

In the guinea pig, we have demonstrated that IL-5 induces selective migration and activation of eosinophils and a pronounced airway hyperresponsiveness to histamine *in vivo*. Similar results were also obtained by other investigators (11, 13, 30). This relatively simple model of IL-5-induced airway hyperresponsiveness enables us to investigate the correlation between eosinophil infiltration and the development of bronchial hyperresponsiveness. This is in contrast to animal models for asthma such as ovalbumin-sensitized and -challenged guinea pigs, where besides the eosinophil a whole range of inflammatory cells are activated (4).

In the airways, it has been well recognized that NANC nerves form part of the local nervous system. Neurogenic inflammation, which is mediated by NANC nerves, may play a

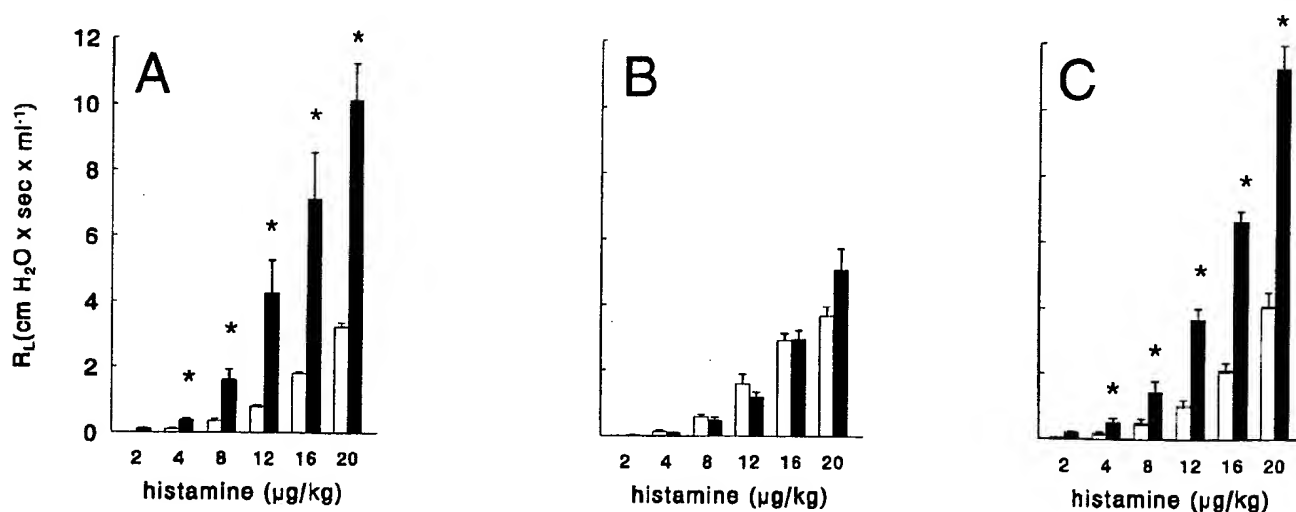
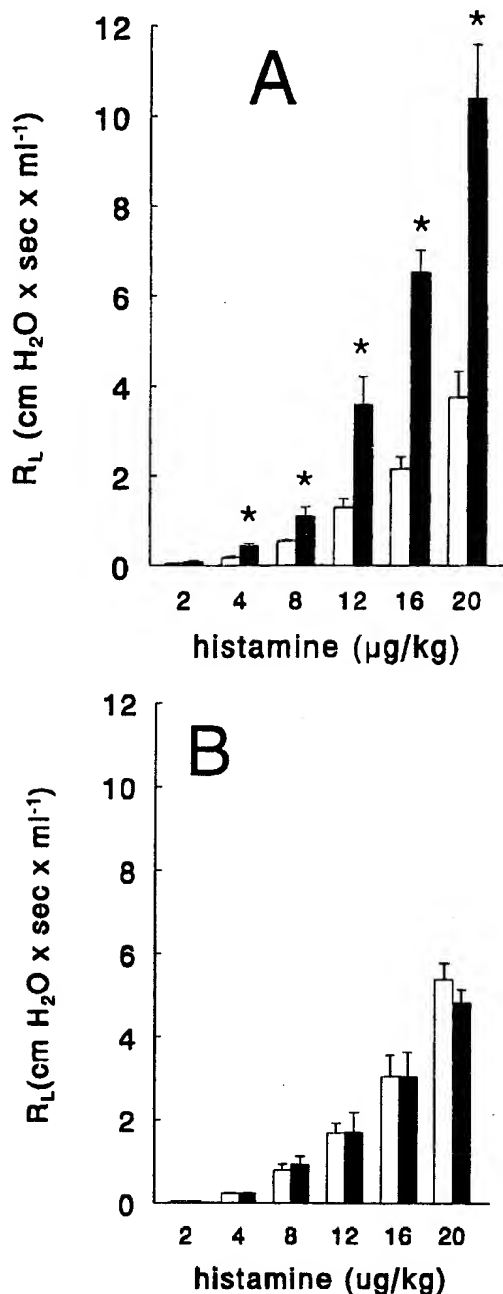
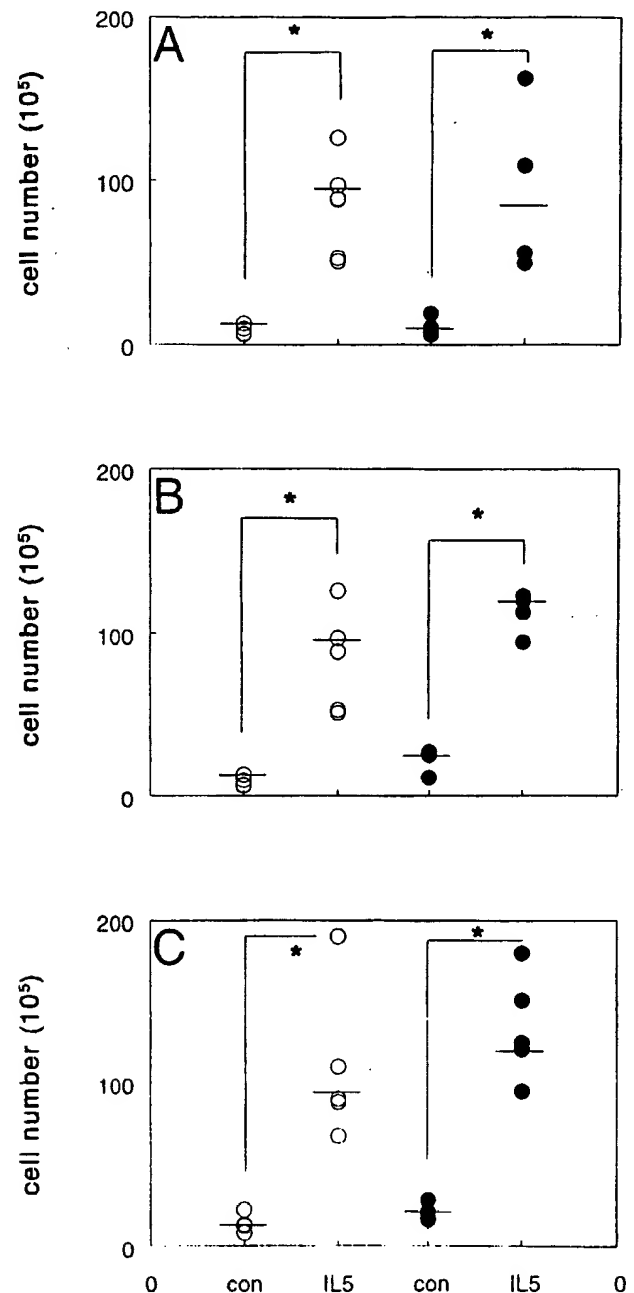


Figure 3. Increase in lung resistance ( $R_L$ ) after intravenous administration of histamine to guinea pigs one day after two intranasal administrations of vehicle (open bars) or  $1 \mu\text{g}$  IL-5 (closed bars) in animals pretreated with (A) vehicle,  $100 \mu\text{l}$  0.1% ethanol, (B) the non-selective neurokinin receptor antagonist, FK224,  $1 \mu\text{mol/kg}$  body weight or (C) the neurokinin-1 receptor antagonist, FK888,  $2 \mu\text{mol/kg}$  body weight.  $n = 5$  animals per group. \* $p < 0.05$  as compared with vehicle-treated animals, ANOVA, Bonferonni test.



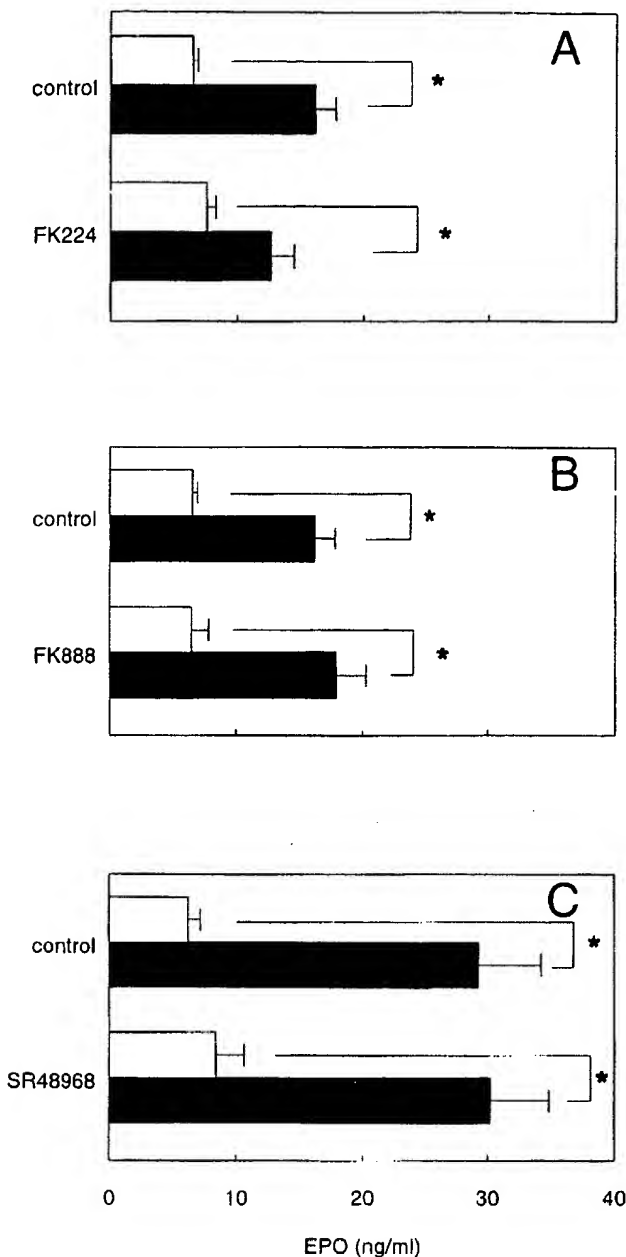
**Figure 4.** Increase in lung resistance ( $R_L$ ) after intravenous administration of histamine to guinea pigs one day after two intranasal administrations of vehicle (open bars) or 1 µg IL-5 (closed bars) in animals pretreated with (A) vehicle, saline, or (B) the neurokinin-2 receptor antagonist, SR 48968, 1 mg/kg body weight.  $n = 5$  animals per group. \* $p < 0.05$  as compared with vehicle-treated animals, ANOVA, Bonferroni test.

role in asthma (15). It is now known that NANC neuropeptides, such as the tachykinins, substance P or neurokinin A, have potent inflammatory effects and can affect airway function in a way that resembles features found in the pathogenesis of asthma. In several animal species, the release of endogenous neuropeptides, elicited by inhalation of capsaicin aerosols, as well as inhaled or infused exogenous tachykinins



**Figure 5.** The total number of eosinophils ( $\times 10^5$  cells) in bronchoalveolar lavage fluid of IL-5- or vehicle-treated guinea pigs (con) after pretreatment with (A) the non-selective neurokinin receptor antagonist, FK224, 1 µmol/kg body weight, or (B) the neurokinin-1 receptor antagonist, FK888, 2 µmol/kg body weight, or (C) the neurokinin-2 receptor antagonist, SR 48968, 1 mg/kg body weight. The guinea pigs received two intranasal administrations of vehicle or IL-5 one day before the measurements. Closed circles represent neurokinin receptor antagonist-pretreated animals whereas open circles represent vehicle-pretreated animals.  $n = 5$  guinea pigs per group. \* $p < 0.05$  as compared with vehicle-treated animals, Kruskal-Wallis one-way analysis of variance.

are able to induce bronchoconstriction and airway hyperresponsiveness to contractile agents (16–18). Immunohistochemical studies of neuronal substance P in airways of asthmatic subjects have yielded conflicting results. While in some studies in



**Figure 6.** Eosinophil peroxidase (EPO) activity (ng/ml) in cell free BAL fluid (cell-free BALF) obtained from guinea pigs one day after two intranasal treatments with vehicle ( $n = 5$ , open bars), or 1  $\mu$ g IL-5 ( $n = 5$ , closed bars) in animals pretreated with (A) the non-selective neurokinin receptor antagonist, FK224, 1  $\mu$ mol/kg body weight, or (B) the neurokinin-1 receptor antagonist, FK888, 2  $\mu$ mol/kg body weight, or (C) the neurokinin-2 receptor antagonist, SR 48968, 1 mg/kg body weight.  $n = 5$  animals per group. \* $p < 0.05$  as compared with vehicle treated animals, ANOVA, Bonferonni test.

asthmatic patients, an increase in both number and length of tachykinin-immunoreactive nerve fibers was found in the airways when compared with nonasthmatic subjects (31, 32), others detected significantly less substance P-like immunoreactivity in lung tissue from asthmatic than from nonasthmatic

patients (33, 34). However, this latter finding may reflect augmented substance P release. Moreover, in asthma patients substance P enhances maximal airway narrowing to methacholine in patients 24 h after inhalation (35).

In the guinea pig, several investigators have demonstrated that depletion of NANC neuropeptides by chronic capsaicin pretreatment prevents the development of allergen-induced hyperresponsiveness despite the presence of eosinophils (19, 20), while others have not (36, 37). In addition, Fischer and coworkers have shown that sensitization of guinea pigs to ovalbumin induced a 1 to 5-fold increase of neuropeptide concentration in lung tissue and also increased 2-fold tachykinin-immunoreactive neurons 24 h after the challenge (38). Van Oosterhout and colleagues reported that in the guinea pig after *in vivo* administration of either IL-5 or substance P, tracheal responses to histamine were significantly increased *in vitro* (13). These results are in accord with our findings with IL-5 or substance P on *in vivo* airway function. In contrast to the IL-5-induced hyperresponsiveness, the substance P-induced hyperresponsiveness was not accompanied by changes in the number of eosinophils in BAL fluid. In addition, substance P did not induce eosinophil degranulation since the EPO activity in cell free BAL fluid was not altered. Since the IL-5 and substance P induce airway hyperresponsiveness to a similar extent *in vivo* after intra-airway administration (this study) and since both responses *in vitro* were not additive (13), the mechanism of action of the two agents may be similar. All these data suggest that IL-5 is important in the recruitment and activation of eosinophils, whereas tachykinins seem to be involved more downstream in the sequence by which eosinophils induce hyperresponsiveness. In this study, the role of neuropeptides in the IL-5-induced airway hyperresponsiveness and eosinophilia was examined using selective neurokinin receptor antagonists. Three types of tachykinin receptors have been characterized namely NK1, NK2, and NK3 (15). These receptors are preferentially activated by substance P, neurokinin A, and neurokinin B, respectively. The tachykinin receptor antagonists studied were: NK1/NK2-receptor antagonist, FK224, and NK1-receptor antagonist, FK888, and NK2-receptor antagonist, SR48968. Our results demonstrate that in the guinea pig the NK2 receptor mediates the hyperresponsiveness found 24 h after intranasal IL-5 administration and that neither the NK1 nor the NK2 receptor is involved in the IL-5-induced eosinophil migration and activation. In addition, the tachykinin substance P was not able to induce eosinophilia in the guinea pig airways. Foulon and colleagues demonstrated that in the guinea pig, NKA and substance P produce bronchoconstriction through NK2 and NK1 receptor stimulation, respectively (39). A role for NK2 receptor in the development of bronchial hyperresponsiveness is further supported by studies in guinea pig and man showing that neurokinin A is far more potent than substance P in contracting airway smooth muscle (21, 40–42). In addition, in lung samples containing membranous airways, NK2-receptor mRNA expression was increased fourfold in asthmatics compared with controls, whereas NK1 receptor mRNA levels were similar in the two groups (43), suggesting that the NK2 receptor could play an important role in the development of bronchial hyperresponsiveness.

Several explanations can be suggested to explain the link between the role of eosinophils and tachykinins in the development of IL-5-induced hyperresponsiveness in the guinea pig. First, IL-5 could induce bronchial hyperresponsiveness via a direct action on sensory nerve endings, completely independent of eosinophil migration/activation. Recently, however, we have demonstrated that for the development of IL-5-

induced airway hyperresponsiveness in the guinea pig the VLA-4 dependent infiltration of eosinophils is essential (14).

Second, tachykinins may be involved in the activation of eosinophils that are already primed by IL-5. Indeed, Kroegel and colleagues have demonstrated that substance P can induce EPO release from isolated guinea pig eosinophils (44). This tachykinin-induced EPO release, however, was not mediated via a neurokinin receptor-dependent mechanism. For this reason, it is not surprising that in our study the selective neurokinin receptor antagonists did not have any effect on the eosinophil activation after IL-5 administration. In addition, in our study, intra-airway application of substance P did not result in eosinophil activation *in vivo* indicating a more downstream role for tachykinins in the development of airway hyperresponsiveness.

Third, it could be possible that mediators from IL-5-activated eosinophils may induce the release of neuropeptides from sensory nerves in the airways. Subsequently, substance P or neurokinin A may induce airway hyperresponsiveness via the NK2 receptor on bronchial smooth muscle cells. Indeed, Garland and colleagues have demonstrated that mediator release from activated eosinophils can directly stimulate tachykinin release from sensory C-fiber neurons in cell culture (45). Released cationic granule proteins from eosinophils are likely mediators, since it has been shown that they can induce the release of neuropeptides in human bronchi, indicating the activation of NANC nerves (46). In addition, intratracheal instillation of cationic proteins induced airway hyperactivity in rats (47). This change in airway function could be antagonized with a neurokinin receptor antagonists. Moreover, it has recently been shown that NK2-receptor antagonist, SR 48968, prevents ovalbumin-induced airway hyperactivity in sensitized guinea pigs (21). In line with these data, it has been demonstrated that airway nerves are surrounded by and infiltrated with eosinophils after ovalbumin challenge (48). These studies together with our results are consistent with an effect of eosinophils on airway neural function.

In conclusion, IL-5 may be an inflammatory mediator primarily involved in the recruitment and activation of eosinophils and the tachykinins, substance P or neurokinin A, have a more downstream role in the sequence by which eosinophils induce airway hyperresponsiveness. Interestingly, in several animal models of asthma, such as ovalbumin-, ozone- and dinitrobenzene-induced hypersensitivity and respiratory viral infections, the development of airway hyperresponsiveness is also dependent on the release of sensory neuropeptides indicating that neuropeptides seem to be a common step in the pathway leading to airway hyperresponsiveness (20, 49–51). Selective neurokinin receptor antagonists or drugs that inhibit the release of neuropeptides could be expected to have a beneficial effect in the pathogenesis of asthma by reducing the neurogenic component of inflammation. However, the future therapeutic potential of such pharmacological agents remains to be investigated in the clinic.

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# A Neurokinin 1-Receptor Antagonist Improves Exercise-induced Airway Narrowing in Asthmatic Patients

MASAKAZU ICHINOSE, MOTOHIKO MIURA, HIDEYUKI YAMAUCHI, NATSUKO KAGEYAMA, MASAFUMI TOMAKI, TATSUYA OYAKE, YUZURU OHUCHI, WATARU HIDA, HIROSHI MIKI, GEN TAMURA, and KUNIO SHIRATO

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Recent reports suggest the involvement of vascular phenomena in exercise-induced asthma. Sensory neuropeptides, such as substance P (SP), which causes airway vascular dilatation and plasma leakage, have been demonstrated to play a role in hyperpnea-induced airway narrowing in animal studies. The purpose of this study was to investigate the importance of tachykinins in exercise-induced airway narrowing in patients with asthma using a selective neurokinin 1-receptor (NK<sub>1</sub>-receptor) antagonist, FK-888. In a double-blind, placebo-controlled, crossover trial, nine subjects with stable asthma were given FK-888 (2.5 mg) or placebo by inhalation 20 min before each exercise at a level previously demonstrated to cause a fall of at least 40% in specific airway conduction (SGaw). Inhalation of FK-888 had no significant effect on baseline SGaw. While the recovery from exercise-induced airway narrowing was significantly faster after treatment with FK-888, the area under the curve for SGaw during the 50 min after exercise was significantly reduced ( $p < 0.05$ ) and the time taken for the SGaw to recover to within 65% of baseline after exercise was also significantly shorter with FK-888 than with placebo ( $p < 0.05$ ). However, treatment with FK-888 did not significantly attenuate the maximal fall in SGaw. These results suggest that NK<sub>1</sub>-receptor-mediated mechanisms are involved in the recovery phase of exercise-induced airway narrowing. The possible mechanisms of these phenomena are discussed. **Ichinose M, Miura M, Yamauchi H, Kageyama N, Tomaki M, Oyake T, Ohuchi Y, Hida W, Miki H, Tamura G, Shirato K. A neurokinin 1-receptor antagonist improves exercise-induced airway narrowing in asthmatic patients.**

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Exercise is a stimulus that can cause airway narrowing in 40-90% of patients with asthma (1-3). It is generally accepted that the pathogenesis of exercise-induced asthma is closely associated with the flux in temperature and moisture that develop within the tracheobronchial tree during the warming and humidification of large volumes of air, although it is not fully known how intra-airway thermal fluxes produce bronchial narrowing (1, 4, 5).

Guinea pigs exhibit dry gas hyperpnea-induced airway narrowing that phenomenologically resembles exercise-induced asthma in human subjects (6, 7). In that model, hyperpnea-induced airway narrowing is ablated after chronic capsaicin pretreatment, which depletes sensory C-fibers of their neuropeptides, and is potentiated by administration of phosphoramidon, which inhibits neutral endopeptidase, the enzyme principally responsible for cleavage and inactivation of sensory neuropeptides in the airways, indicating that tachykinins released from sensory nerve end-

ings may be involved in the response (8). There is a hypothesis that exercise-induced asthma is, at least in part, due to vascular phenomena such as vascular engorgement and plasma leakage that could thicken the mucosa and thereby narrow airway diameters, which could amplify the effects of airway smooth muscle contraction (9, 10). Sensory neuropeptides, especially tachykinins such as substance P (SP), can bring about both vascular dilation and microvascular leakage via neurokinin 1 (NK<sub>1</sub>)-receptors (11). Thus, tachykinins are likely to be involved in the exercise-induced airway narrowing, although the role of endogenously released tachykinins in exercise-induced airway narrowing in asthmatic subjects has not been explored due to the scarcity of suitable antagonists for tachykinins.

FK-888 ( $N^2$ -[(4*R*)-4-hydroxy-1-(1-methyl-1*H*-indol-3-yl)carbonyl-L-prolyl]-*N*-methyl-*N*-phenylmethyl-3(2-naphthyl)-L-alaninamide], Fujisawa Pharmaceutical Co., Ltd.) is a novel NK<sub>1</sub>-receptor selective antagonist (12, 13). Receptor-binding assays have shown that FK-888 binds competitively to SP binding sites on guinea pig lung membranes and rat brain cortical synaptic membranes, but it has no effect on leukotriene D<sub>4</sub>, bradykinin, cholecystokinin, angiotensin II, vasopressin, or endothelin-1 binding sites; thus, it is selective for tachykinin receptors (13). In the NK<sub>1</sub>-receptor bioassay (contraction of guinea pig ileum by SP), FK-888 exhibited high affinity with a pA<sub>2</sub> value of 9.29. FK-888 has at least 10,000 times higher affinity for the guinea pig ileum NK<sub>1</sub>-receptor than for the rat NK<sub>2</sub> (contraction of vas deferens

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Am J Respir Crit Care Med Vol 153. pp 936-941, 1996

by neurokinin A [NKA]) and rat NK<sub>3</sub> (contraction of portal vein by neurokinin B [NKB]) receptors (13). Thus, using this selective compound, we can observe the NK<sub>1</sub>-receptor-mediated responses in human beings.

Our aim was to study the involvement of endogenous tachykinins in exercise-induced airway narrowing in patients with asthma by means of this NK<sub>1</sub>-receptor antagonist. We tested the effect of FK-888 on airway narrowing after exercise challenge in a double-blind, placebo-controlled, crossover trial.

## METHODS

### Subjects and First Exercise Test

Twelve subjects with asthma who satisfied the American Thoracic Society criteria for asthma (14) (10 men, two women), aged 18–50 yr, were tested for exercise-induced airway narrowing. Exercise was performed on a bicycle ergometer (Type 18071; Gould Godart BV, Bilthoven, the Netherlands). During exercise, the subjects breathed dry air at room temperature (20–23°C) through a face mask (Stress Test Mask; Respironics Inc., Monroeville, PA) from a Douglas bag reservoir. Ventilation and oxygen consumption were measured with an aerobics processor (Type 391; NEC San-ei Co., Tokyo, Japan), and the heart rate was monitored continuously by three-lead electrocardiography. Exercise challenge was carried out by gradually increasing the workload at 25 watts/15 s until 85% of the predicted maximal heart rate was achieved. The subjects were then asked to continue exercising at that workload for 6 min. Nine subjects (eight male, one female) aged 18–43 yr with at least a 40% fall in the specific airway conductance (SGaw) were enrolled in the study. The baseline lung function of each patient is shown in Table 1. Subject 3 was treated with inhaled  $\beta_2$ -agonist and oral theophylline. Subject 9 was treated with inhaled  $\beta_2$ -agonist. The other seven patients were free from any medication. Bronchodilator therapy was withheld for at least 24 h and caffeine-containing drinks were not allowed for 12 h before exercise challenge. Written informed consent for the protocol, which was reviewed and approved by the Tohoku University Committee on Clinical Investigation, was obtained from each patient.

### Protocol

Pulmonary function was assessed with a spirometer, and airway resistance and thoracic gas volume were measured in a constant-volume, pressure-compensated, whole-body plethysmograph (2800J Autobox; Gould Electronics, Dayton, OH) with the subject panting at a frequency of 2 Hz and a peak-to-peak flow of 2–3 L/s. The signals were automatically converted to mean values for airway resistance, thoracic gas volume, and specific airway conductance (SGaw) by computer as previously described (15).

The second and third exercise tests were carried out at least 4 d apart to eliminate any possibility of carryover effects. On each day, SGaw was measured and then the subjects inhaled FK-888 (2.5 mg; Fujisawa Pharmaceutical Company, Osaka, Japan) or placebo (FK-888 vehicle: lactose) in powder form randomly using a spinhaler. Twenty minutes later, SGaw (baseline value) was remeasured and exercise challenge, the workload of which was determined on the first exercise test day, was done on the second and third exercise test days.

Airway response to exercise was expressed as the percentage change in SGaw from the mean baseline value against time. SGaw was measured at 1-min intervals at 2 to 10 min after exercise and every 5 min at 10 to 50 min after exercise.

### Statistical Analysis

Data are expressed as mean  $\pm$  SEM. The airway narrowing after exercise was evaluated as the maximal percent change in SGaw from the baseline value and the area under the curve (AUC) of the percentage fall in baseline SGaw time course over 50 min. Because the proposed minimal change in SGaw after bronchial provocation challenge differs by 35% from baseline value (16), we calculated the time required to recover to within 65% of baseline values (recovery time). In one subject in whom the SGaw did not return to within 65% of the baseline value after 50 min, 50 min was used as the recovery time. Wilcoxon signed rank test was used to compare maximal fall in percent SGaw, AUC, and recovery time between the values with placebo and those with FK-888,

TABLE 1  
PULMONARY FUNCTION AT BASELINE IN 9 SUBJECTS\*

	Mean Value	Percentage of Predicted Value
FVC, liters	4.96 $\pm$ 0.17	107.1 $\pm$ 3.6
FEV <sub>1</sub> , liters	3.91 $\pm$ 0.21	103.7 $\pm$ 2.0
FEV <sub>1</sub> /FVC, %	78.5 $\pm$ 2.0	
Total lung capacity, liters	6.87 $\pm$ 0.29	110.4 $\pm$ 3.6
Residual volume, liters	1.86 $\pm$ 0.11	116.9 $\pm$ 8.7

\* Values are means  $\pm$  SEM.

and to compare heart rate, minute ventilation, and oxygen consumption at rest and during exercise between the values of the two exercise tests. A p value of less than 5% was considered to indicate significance.

## RESULTS

The initial values for SGaw before placebo or FK-888 on the second and third study days were comparable, and neither placebo nor FK-888 had a significant effect on airway caliber (baseline values) (Table 2).

The exercise workload was kept the same on the two study days. In all nine subjects, there was no significant difference in the heart rate, minute ventilation, and oxygen consumption at rest and during exercise between the values with placebo treatment and FK-888 treatment (Table 3).

The SGaw percent fall after exercise of each subject is shown in Figure 1. On the placebo treated days, exercise caused SGaw to fall immediately (reached maximal fall within 2 to 15 min) and recovered to baseline values gradually. On the FK-888 treated days, the SGaw also decreased promptly and reached maximal at 4 to 10 min. The mean maximal percent decrease in SGaw after exercise in the subjects given placebo was 40.5  $\pm$  2.2% of baseline values; in the subjects treated with FK-888, it was 45.7  $\pm$  4.7% of baseline values. There was no significant difference between the values ( $p = 0.236$ ). However, the recovery from exercise-induced airway narrowing was faster on the FK-888 treated days than on the placebo treated days; the mean recovery time for 65% of the baseline SGaw was 25.0  $\pm$  4.1 min after the placebo and 15.9  $\pm$  3.7 min after FK-888 ( $p < 0.05$ ) (Figure 2). Treatment with FK-888 also significantly improved the AUC after exercise;  $-1,540 \pm 152\%$  min after placebo and  $-1,123 \pm 210\%$  min after FK-888 ( $p < 0.05$ ) (Figure 3).

## DISCUSSION

The present study demonstrates that an NK<sub>1</sub>-receptor selective

TABLE 2  
SPECIFIC AIRWAY CONDUCTANCE (SGaw) BEFORE EXERCISE (PER SECOND PER CM H<sub>2</sub>O)

	Placebo		FK-888	
	Before	After	Before	After
1	0.324	0.324	0.303	0.351
2	0.357	0.351	0.364	0.362
3	0.211	0.254	0.147	0.160
4	0.448	0.429	0.397	0.319
5	0.290	0.351	0.256	0.265
6	0.386	0.396	0.343	0.316
7	0.220	0.242	0.197	0.183
8	0.172	0.184	0.190	0.169
9	0.184	0.180	0.186	0.188
Mean	0.288	0.301	0.265	0.257
SEM	0.033	0.030	0.030	0.028

TABLE 3  
HEART RATE, MINUTE VENTILATION, AND OXYGEN  
CONSUMPTION DURING ADMINISTRATION  
OF THE STUDY AGENTS\*

Agent	Heart Rate (beats/min)	Minute Ventilation (L/min)	Oxygen Consumption (ml/min)
At rest			
Placebo	71.0 ± 1.9	9.0 ± 0.8	283 ± 15
FK-888	74.3 ± 2.5	8.7 ± 0.6	307 ± 22
During exercise			
Placebo	160.6 ± 4.5	68.6 ± 6.5	2,406 ± 128
FK-888	152.3 ± 3.7	67.4 ± 6.3	2,401 ± 165

\* Values are means ± SEM.

antagonist significantly improves the recovery phase of exercise-induced airway narrowing, suggesting that NK<sub>1</sub>-receptors that seem to be stimulated by endogenously released tachykinins are involved in the phenomenon.

The pathogenesis of exercise-induced asthma is thought to be closely associated with the intra-airway thermal fluxes caused by the rapid large volume ventilation (1), although it is not known how the thermal fluxes cause airway narrowing. One hypothesis is that it is caused by an inflammatory mediator mechanism. Rapid breathing may cause evaporation of mucosal surface water and an increase in osmolarity, which then results in mast cell degranulation. Because mast cell-derived mediators, such as histamine and leukotrienes, can cause airway smooth muscle contraction, airway vascular dilatation, and microvascular leakage

(17), it is possible that the airway narrowing after exercise is due to these mediators. The effectiveness of a mast cell stabilizer, cromolyn (2, 18, 19), and the antagonists or synthesis inhibitor of mast cell-derived mediators, such as histamine (20) and sulfidopeptide leukotrienes (21, 22), supports this hypothesis.

Neural mechanisms constitute an alternative explanation for the exercise-induced airway narrowing. There are two excitatory nervous pathways, namely cholinergic and nonadrenergic noncholinergic nervous systems (23), which may be involved in the response. A change in the mucosal surface osmolarity by hyperventilation during exercise may stimulate airway sensory nerve endings and result in reflex activation of the two nervous systems. In addition, histamine and leukotrienes, which may be released as mentioned previously, could also activate both the cholinergic and excitatory nonadrenergic noncholinergic pathways by sensory nerve stimulation (23). Several reports have shown that anticholinergic agent inhalation somewhat reduces the severity of exercise-induced airway narrowing (24–26), suggesting the partial involvement of the cholinergic mechanism in the response. The possibility of an excitatory nonadrenergic noncholinergic mechanism, which involves the release of neuropeptides from airway sensory nerves, in exercise-induced airway narrowing has been suggested by an animal experiment (8). In this experiment, it was shown that hyperpnea increases pulmonary resistance in guinea pigs. This response is markedly reduced by capsaicin pretreatment, which causes sensory neuropeptide depletion and is potentiated by administration of inhibitors of neutral endopeptidase, the enzyme responsible for cleavage and inactivation of the peptides, suggesting that tachykinin release from airway sensory nerves is an important component of hyperpnea-induced airway narrowing. Furthermore, in guinea pigs, dry gas

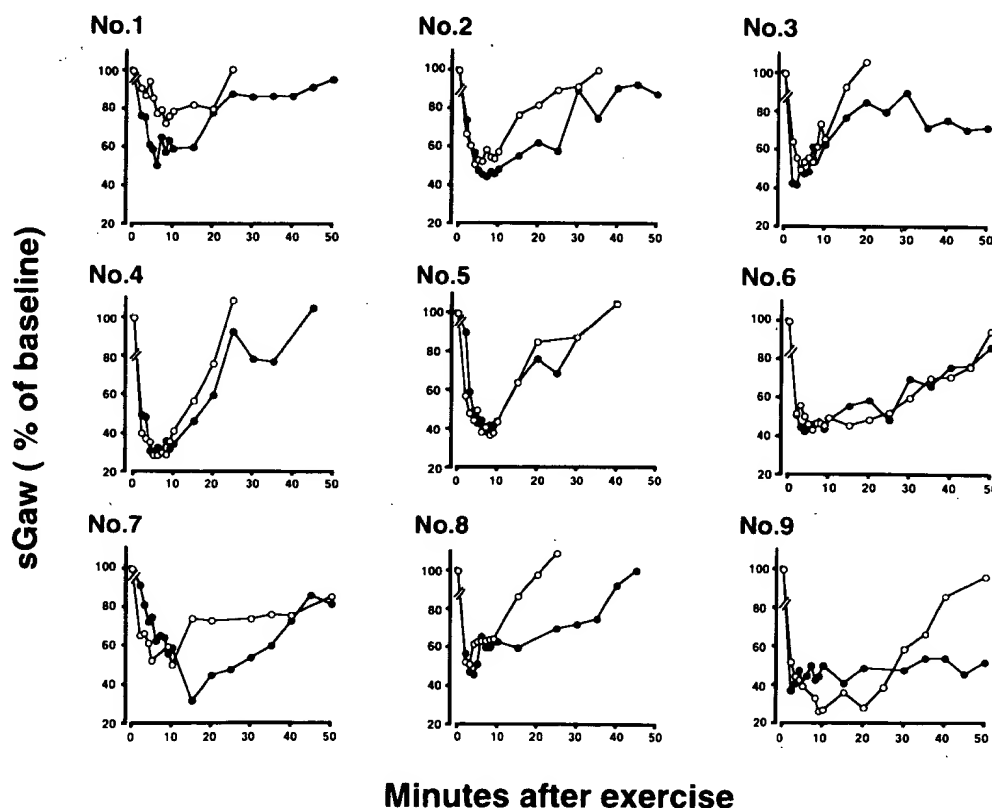


Figure 1. Percent changes in specific airway conductance (SGaw) over time after exercise, after treatment with FK-888 (open circles), or placebo (solid circles) in each subject.

RECOVERY TIME  
(min)

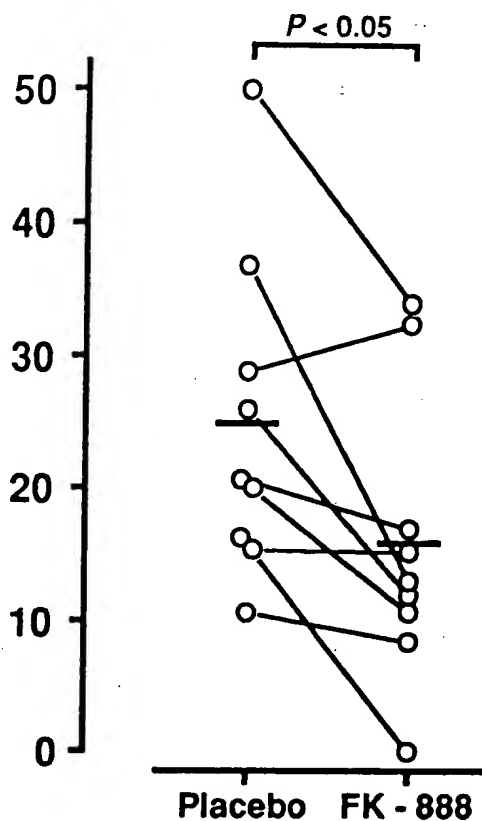


Figure 2. Time required for the specific airway conductance (SGaw) to recover to within 65% of baseline (recovery time) after treatment with FK-888 or placebo.

sGaw AREA  
UNDER THE CURVE  
( - % min)

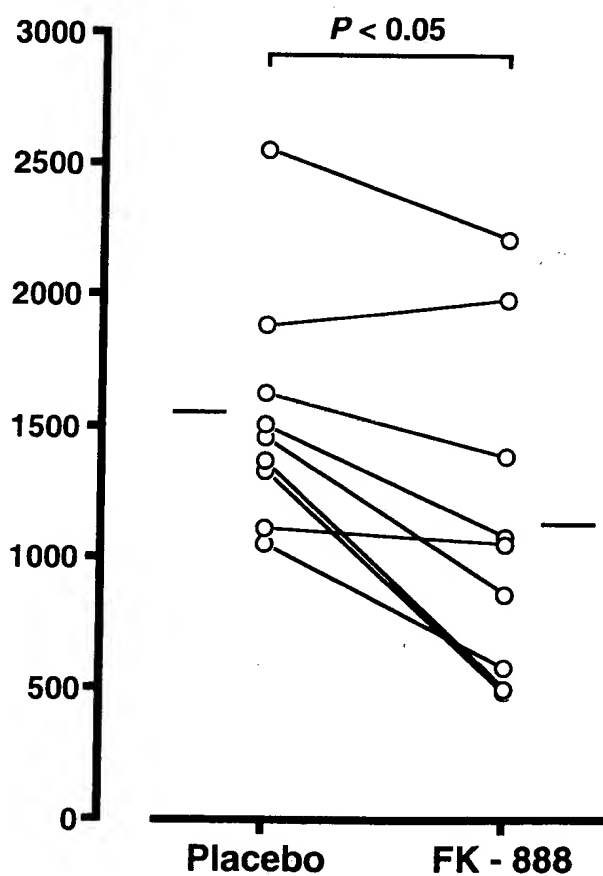


Figure 3. Area under the curve of the percentage fall of SGaw after exercise with placebo or FK-888. Bars indicate the mean values.

hyperpnea causes bronchovascular hyperpermeability, and this response is reduced by capsaicin pretreatment, indicating that endogenously released tachykinins are also involved in the vascular events (27). Recently, there is more direct evidence that the physical sequelae of dry gas hyperpnea, hyperosmolarity but not hypothermia, stimulates tachykinin release from neonatal rat dorsal root ganglion cells (C-fiber neurons) in primary culture (28).

Three kinds of tachykinin receptor subtypes, namely NK<sub>1</sub>-, NK<sub>2</sub>-, and NK<sub>3</sub>-receptors, have been characterized. In the airways, only NK<sub>1</sub>- and NK<sub>2</sub>-receptor mediated responses are important (11). In human airway smooth muscle contraction, neurokinin A (NKA) is more potent than SP. In addition, NK<sub>1</sub>-selective compounds, [Pro<sup>7</sup>] SP (1-11) sulfone and [β-Ala<sup>4</sup>, Sar<sup>9</sup>] SP (4-11) sulfone, do not induce significant airway smooth muscle contraction up to 10<sup>-5</sup> M, and the selective agonist for the NK<sub>3</sub>-receptor, [Me Phe<sup>7</sup>] NKB (4-10), is also almost inactive. However, the NK<sub>2</sub>-selective fragment [Nle<sup>10</sup>] NKA (4-10) causes a potent airway smooth muscle contraction. This evidence suggests the major involvement of NK<sub>2</sub>-receptors in the airway smooth muscle contractile response (29). On the other hand, NK<sub>1</sub>-receptor-mediated airway responses are vasodilation, microvascular leakage, and secretion (11). However, because until now a tachykinin receptor antagonist was not available for human use, the role of tachykinins in exercise-induced airway response in patients with asthma has not yet been explored.

FK-888 is a potent NK<sub>1</sub>-receptor selective antagonist, as mentioned previously (12, 13). In human lungs, the K<sub>i</sub> value of this compound is 0.8 nM, which is similar to the most potent endogenous NK<sub>1</sub>-receptor stimulant SP (30). In the present study, we used 2.5 mg of FK-888. At this dose, the plasma concentration of this compound at 20 to 70 min after FK-888 inhalation is 10<sup>-6</sup> to 3 × 10<sup>-6</sup> M (Fujisawa Pharmaceutical Company's data). Therefore, we think that 2.5 mg of FK-888 inhalation is adequate to block the NK<sub>1</sub>-receptor function in the airways. Of course, it is possible that complete blocking of the NK<sub>1</sub>-receptors may require a higher dose.

In the present study, NK<sub>1</sub>-receptor antagonism by FK-888 improved both the AUC and recovery time after exercise without attenuating the maximal airway narrowing after exercise. This is the first report examining the effectiveness of a tachykinin receptor antagonist on exercise-induced responses in human airways. In guinea pig airways, it has been shown that NK<sub>1</sub> selective antagonist CP-96,345 reduces the magnitude of dry gas hyperpnea-induced increase in pulmonary resistance by one half, and NK<sub>2</sub> selective antagonist SR-48,968 blocks the response more completely, suggesting that both NK<sub>1</sub>- and NK<sub>2</sub>-receptors participate in the airway narrowing after exercise (31). On the contrary, hyperpnea-mediated bronchovascular hyperpermeability was reduced by neither NK<sub>1</sub>- nor NK<sub>2</sub>-receptor antagonists (31). In this species, both of these receptor stimulations cause airway smooth muscle contraction, with a higher affinity to NK<sub>2</sub>- than to NK<sub>1</sub>-receptors (32), indicating that tachykinin causes hyperpnea-induced airway narrowing via airway smooth muscle contraction rather than via bronchovascular leakage. However, in human airways, NK<sub>1</sub>-receptor stimulation does not cause airway smooth muscle contraction (29). Taken together, the inhibitory effect of FK-888 observed in our study suggests that NK<sub>1</sub> receptor-mediated vascular phenomena such as vascular engorgement, plasma leakage, and airway wall edema contribute to the prolonged airway narrowing phase rather than to the acute onset phase, which seems to be caused by airway smooth muscle contraction.

The importance of the vascular phenomenon in the exercise-induced airway narrowing was strongly suggested by McFadden (9, 10). Typically, two or three arteries wind around the walls of the airways and anastomose freely with each other to form

a plexus in the peribronchial space; arterial branches also penetrate the muscular layer and form a second plexus in the submucosa. Thus, two highly interconnected capillary networks line and cover the tracheobronchial tree from the central airways to the terminal bronchioles (9). Furthermore, the tracheobronchial capillary bed is hypertrophic and hyperplastic in patients with asthma compared with other subjects (33). Then, rapid expansion of the blood volume in peribronchial vascular plexi, capillary leakage, and mucosal airway wall edema formation may contribute to the airway narrowing after exercise, because histamine, leukotrienes, and SP, which are thought to be released after exercise, have such vascular effects.

In summary, we have demonstrated that the NK<sub>1</sub>-receptor selective antagonist, FK-888, attenuates the recovery phase of exercise-induced airway narrowing, indicating that NK<sub>1</sub>-receptors are involved in the response. Because in human airways NK<sub>1</sub>-receptor-mediated responses are dominant in vasculature compared with airway smooth muscle (11, 29), we suggest that the effect of NK<sub>1</sub>-receptor antagonism on exercise-induced airway narrowing is possibly due to the inhibition of vascular engorgement and/or capillary leakage (and subsequent airway wall edema) rather than to a reduction in the airway smooth muscle contraction.

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## The effect of the NK2 tachykinin receptor antagonist SR 48968 (saredutant) on neurokinin A-induced bronchoconstriction in asthmatics

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*The effect of the NK2 tachykinin receptor antagonist SR 48968 (saredutant) on neurokinin A-induced bronchoconstriction in asthmatics. J. Van Schoor, G.F. Joos, B.L. Chasson, R.J. Brouard, R.A. Pauwels. ©ERS Journals Ltd 1998.*

**ABSTRACT:** Inhalation of neurokinin (NK) A causes bronchoconstriction in patients with asthma. The NKA-induced bronchoconstriction in isolated human airways is mediated via the NK2 receptor and inhibited by SR 48968, a potent and specific non-peptide tachykinin NK2 receptor antagonist. In the present study, the effect of orally administered SR 48968 on NKA-induced bronchoconstriction was examined in 12 mild asthmatics.

On the screening day and during the study periods, increasing concentrations of NKA ( $3.3 \times 10^{-3}$  to  $1.0 \times 10^{-4}$  mol·mL<sup>-1</sup>) were inhaled, until the forced expiratory volume in one second (FEV<sub>1</sub>) and specific airway conductance (sGaw) decreased by at least 20 and 50%, respectively. During the study periods, 100 mg SR 48968 or matched placebo was ingested in a double-blind, randomized, crossover fashion and NKA provocation was performed at 1.5 and 24 h after dosing.

At 1.5 h, the mean (SEM) log<sub>10</sub> provocative concentration of NKA causing a 20% fall in FEV<sub>1</sub> (PC<sub>20</sub> FEV<sub>1</sub>) was -6.25 (0.20) after SR 48968 and -6.75 (0.17) after placebo ( $p=0.05$ ); the mean log<sub>10</sub> provocative concentration of NKA causing a 35% fall in sGaw (PC<sub>35</sub> sGaw) was -7.02 (0.28) after SR 48968 and -7.64 (0.19) after placebo ( $p=0.05$ ). At 24 h, the mean log<sub>10</sub> PC<sub>20</sub> FEV<sub>1</sub> was -6.21 (0.17) after SR 48968 and -6.65 (0.11) after placebo ( $p=0.05$ ); the mean log<sub>10</sub> PC<sub>35</sub> sGaw was -6.85 (0.23) after SR 48968 and -7.17 (0.15) after placebo (nonsignificant). As PC<sub>20</sub> FEV<sub>1</sub> and/or PC<sub>35</sub> sGaw were not reached in up to 4 patients per SR 48968 group, the differences between SR 48968 and placebo were underestimated.

In conclusion, oral treatment with 100 mg SR 48968 caused a significant inhibition of neurokinin A-induced bronchoconstriction in asthmatics. This finding constitutes the first evidence of inhibition of sensory neuropeptide-induced bronchoconstriction by a selective tachykinin receptor antagonist in humans.

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Substance P (SP) and neurokinin (NK) A are members of the tachykinin peptide family and have been implicated as neurotransmitters which mediate the excitatory part of the nonadrenergic, noncholinergic (e-NANC) nervous system [1-3]. In the human airways, they are contained within sensory unmyelinated C nerve fibres, which are distributed beneath or within the airway epithelium, around blood vessels and glands, within the bronchial smooth muscle layer and around local ganglion cells [4-8]. Recent findings in both experimental animals and humans, however, suggest that non-neural cells (endothelial cells, eosinophils and macrophages), either resident or circulating, can also be a source of tachykinins and that immune stimuli can boost tachykinin production from immunocytes [9]. A reduced SP-like immunoreactivity (SP-LI) content of asthmatic airways compared with nonasthmatic subjects has been reported, suggesting an augmented SP release in asthma [10]. Supporting this hypothesis, bronchoalveolar lavage fluid [11] and induced sputum [12] from asthmatics were found to contain increased amounts of

SP-LI. SP and NKA contract human airways *in vitro* and *in vivo*, NKA being more potent than SP and asthmatics being more sensitive than normal subjects [5, 13-17]. Other potentially important airway effects of tachykinins include mucus secretion, cough, vasodilatation, increased vascular permeability and a broad array of pro-inflammatory effects involving various types of leukocytes [1-3].

SP and NKA interact with their target cells in the airways through specific tachykinin receptors, with SP being the preferential agonist for the tachykinin NK1 receptor and NKA the preferential agonist for the tachykinin NK2 receptor [18]. Increased expression of NK1 [19] and NK2 [20] tachykinin receptor gene messenger ribonucleic acid (mRNA) in asthmatic airways has been reported. In isolated normal human airways *in vitro*, tachykinin-induced bronchoconstriction is mediated predominantly by tachykinin NK2 receptors [8, 21-23]; recently, however, involvement of tachykinin NK1 receptors has also been noted [24, 25]. Tachykinin NK1 receptor stimulation appears to be important in eliciting neurogenic inflammation [1-3, 18].

Because of their presence and release in the airways and their ability to mimic various pathophysiological features of asthma, SP and NKA fulfil two of the three criteria for a presumed mediator of asthma; their pathogenetic role will be defined through the use of tachykinin antagonists in clinical trials of asthmatics.

SR 48968 (saredutant) is a potent ( $pA_{50} = 9.40$  on isolated human bronchus) and selective competitive nonpeptide tachykinin NK2 receptor antagonist [22, 23, 26]. In order to clarify further the *in vivo* mechanisms underlying the NKA-induced bronchoconstriction in patients with asthma, the effect of SR 48968 in asthmatics was studied in a double-blind, randomized and placebo-controlled trial.

## Subjects and methods

### Patients

Twelve male, adult, nonsmoking subjects with stable mild-to-moderate asthma were recruited for the trial. All participants met the American Thoracic Society diagnostic criteria for asthma [27] and no relevant concomitant diseases were present. All patients were atopic.

The only treatment allowed during the study period consisted of inhaled salbutamol. Patients number 1 and 7 were receiving inhaled glucocorticosteroids (budesonide  $800 \mu\text{g}\cdot\text{day}^{-1}$  and  $400 \mu\text{g}\cdot\text{day}^{-1}$ , respectively) and discontinued its use at least 2 weeks before the first screening visit. Their morning baseline forced expiratory volume in one second (FEV<sub>1</sub>) was  $\geq 70\%$  of predicted and the provocative concentration causing a 20% fall in FEV<sub>1</sub> (PC<sub>20</sub> FEV<sub>1</sub>) methacholine was  $< 8 \text{ mg}\cdot\text{mL}^{-1}$ . No patients were studied within 4 weeks of an upper respiratory tract infection or exacerbation of asthma.

The study protocol was approved by the ethical committee of the University Hospital of Ghent. All participants gave their written informed consent.

### Study design

This study was of a randomized, double-blind, placebo-controlled, two-period, crossover design. On the screening day, the patients underwent an NKA inhalation challenge. To be eligible for inclusion in the trial, they had to experience a fall in FEV<sub>1</sub> of at least 10%, compared with their prechallenge value. All screened patients fulfilled this criterion. The screening tests had to be performed within the week prior to the start of the study (study period 1). Both study periods comprised about 25 h, during which the participants were hospitalized in the clinical unit of the Department of Respiratory Diseases, including an overnight stay. The patients arrived at the department at around 08:00 h, following an overnight fast of at least 8 h.

The study medication, 100 mg of SR 48968 ((s)-N-methyl-N-(4-acetylaminophenyl)-2-(3,4-dichlorophenyl) butylbenzamide succinate) or matched placebo (formulated as four capsules), was ingested with 100 mL of tap water. An NKA challenge was performed at 1.5 and 24 h post-dose. The baseline FEV<sub>1</sub> before each NKA challenge had to be within 15% of the baseline FEV<sub>1</sub>, obtained at the screening visit; if this was not the case, the

patient was rescheduled for another visit. Foods and beverages containing caffeine had to be discontinued for at least 24 h before each study period and until the end of each period. Short-acting inhaled  $\beta_2$ -agonists were withheld for at least 10 h before each NKA challenge. Breakfast was served 3 h post-dose, after completing the NKA challenge, while lunch and dinner were served 6 and 12 h post-dose, respectively. The next morning, breakfast was served after completion of the NKA challenge (24 h post-dose), following which the patients were allowed to leave the clinic. They returned 48 h post-dose for a safety control visit (enquiry into adverse events, changes in concomitant medication and blood sampling).

Study period 2 was performed after a wash-out of 3–6 days after the first trial drug ingestion. Patients were requested to avoid all strenuous physical efforts from the week preceding study period 1 until the end of the study.

### Pulmonary function testing

The specific airway conductance (sGaw) was measured with a constant volume body plethysmograph (Jaeger, Würzburg, Germany). sGaw was calculated from airway resistance and thoracic gas volume, using the MasterLab software package (version 3.2, 1991, Jaeger), installed on a personal computer. Each value represents the mean of five consecutive manoeuvres. The FEV<sub>1</sub> was obtained from flow-volume loops, obtained from a pneumotachograph, using the same apparatus and software. The highest value of three consecutive manoeuvres was accepted for evaluation at each performance. sGaw was always measured before the FEV<sub>1</sub>, to avoid changes in airway calibre in response to deep inhalation. All manoeuvres were performed with the patient in the sitting position, the nose occluded by a clip. The same lung function technician and body box were used throughout the study.

### Bronchial challenge tests

The PC<sub>20</sub> for methacholine was determined by measuring the decrease in FEV<sub>1</sub> after inhalation of doubling concentrations of methacholine, according to the method of COCKCROFT *et al.* [28].

NKA was inhaled using a protocol slightly modified from our previous work [15]. Before each NKA inhalation challenge, baseline sGaw and FEV<sub>1</sub> were determined. The patients then inhaled the NKA diluent and sGaw and FEV<sub>1</sub> were measured 3 and 7 min after the start of the inhalation, with the lowest value of each being considered as the postdiluent baseline sGaw and FEV<sub>1</sub>, respectively. The actual NKA challenge was performed provided the FEV<sub>1</sub> did not fall by  $> 10\%$  after inhaling diluent. During the challenge, increasing concentrations of NKA ( $3.3 \times 10^{-9}$ ,  $1.0 \times 10^{-8}$ ,  $3.3 \times 10^{-8}$ ,  $1.0 \times 10^{-7}$ ,  $3.3 \times 10^{-7}$  and  $1.0 \times 10^{-6} \text{ mol}\cdot\text{mL}^{-1}$ ) were inhaled until FEV<sub>1</sub> fell by at least 20% and sGaw decreased by at least 50% of the respective postdiluent baseline values.

NKA was obtained from Peninsula (St Helens, UK) and was diluted in saline containing 1% human serum albumin (Behringwerke, Marburg, Germany). The dilutions of NKA were freshly prepared on the morning of the challenge and kept on ice until nebulization. The aerosols were produced

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using a Mallinckrodt jet nebulizer (Mallinckrodt Diagnostica, Petten, The Netherlands); this method has been validated and described previously [17, 29]. First, a collapsible 30 L plastic bag, which served as a drying chamber, was filled with nitrogen ( $N_2$ ) gas. Then, 0.5 mL of diluent or each subsequent NKA concentration was sprayed by compressed  $N_2$  (400 kPa) in  $60 \pm 10$  s into the drying chamber, in which the droplets evaporated rapidly to dry particles. Finally, the patient inhaled the aerosol from the bag in 2 min by quiet tidal breathing through a three-way valve and a mouthpiece, until the collapse of the bag. Supplementary oxygen (at a flow rate of  $4 \text{ L} \cdot \text{min}^{-1}$ , inspiratory oxygen fraction ( $F_{I,O_2}$ ) = 0.995) was supplied into the mouthpiece. The patient performed the inhalation in the sitting position, with the nose occluded by a clip. Pulmonary function measurements ( $sGaw$  and FEV<sub>1</sub>) were performed at 3 and 7 min after the start of the inhalation of each concentration. The nebulizations of the different concentrations were initiated at 10 min intervals. The NKA challenge was stopped when PC20 FEV<sub>1</sub> NKA and PC35  $sGaw$  NKA could be calculated. A PC20 or PC35 value of  $3.3 \times 10^{-6} \text{ mol} \cdot \text{mL}^{-1}$  (a 0.5 log higher concentration than the highest concentration tested) was attributed arbitrarily in those cases where the desired fall in FEV<sub>1</sub> or  $sGaw$  was not obtained.

#### Plasma for SR 48968 levels

Blood samples for the quantification of SR 48968 plasma concentrations were taken predose and at 1.5 h, 3 h (before breakfast) and 24 h post-dose in each study period. Samples (6 mL) were collected into polypropylene sodium heparin tubes and immediately after sampling, the tubes were centrifuged at  $1500 \times g$  for 10 min. The plasma was then pipetted into polypropylene tubes and stored at  $-20^\circ\text{C}$ , until batch analysis. Quantification of SR 48968 was performed using gas chromatography with electron capture detection. This method has a limit of detection of  $0.5 \text{ ng} \cdot \text{mL}^{-1}$  and a limit of quantification of  $1.0 \text{ ng} \cdot \text{mL}^{-1}$  (data on file at Sanofi).

#### Statistical analysis

Comparisons of the predose baseline FEV<sub>1</sub> values between the SR 48968 group and the placebo group, and of the postdiluent baseline FEV<sub>1</sub> values in these two groups were performed using a Wilcoxon matched-pairs test.

The values of PC20 FEV<sub>1</sub> NKA and PC35  $sGaw$  NKA (expressed in  $\text{mol} \cdot \text{mL}^{-1}$ ) were log<sub>10</sub> transformed; treatment with SR 48968 was compared with placebo, at 1.5 h and 24 h post-dose, by means of a cross-over analysis of variance (ANOVA) test. A  $p$ -value  $\leq 0.05$  was considered significant. Data are expressed as the mean  $\pm$  SEM of the logarithmically transformed values. The relationship between the level of protection and the serum SR 48968 concentration, at 1.5 h and at 24 h post-dose, was calculated using Spearman's rank correlation test. The level of protection at a given time point was defined as the difference between the log<sub>10</sub> PC20 FEV<sub>1</sub> NKA value on the SR 48968 day and that on the placebo day for individual patients.

## Results

### Patients

Twelve patients, aged 19–36 yrs (mean 28.6 yrs), participated in the trial. Their mean ( $\pm$ SEM) baseline FEV<sub>1</sub> was  $4.20 \pm 0.20 \text{ L}$  or  $96.6 \pm 4.4\%$  of predicted. Their mean PC20 FEV<sub>1</sub> methacholine was  $5.2 \pm 0.6 \text{ mg} \cdot \text{mL}^{-1}$  (table 1).

### Effects of SR 48968 on baseline FEV<sub>1</sub>

There were no statistically significant differences between the predose baseline mean ( $\pm$ SEM) FEV<sub>1</sub> values on the SR 48968 ( $4.04 \pm 0.18 \text{ L}$ ) and the placebo day ( $4.03 \pm 0.17 \text{ L}$ ) ( $p=1.000$ ), or between the postdiluent baseline mean ( $\pm$ SEM) FEV<sub>1</sub> values on these two days ( $3.98 \pm 0.18 \text{ L}$  for SR 48968 versus  $3.94 \pm 0.17 \text{ L}$  for placebo) ( $p=0.656$ ).

### Reproducibility of the neurokinin A challenge

At screening, all 12 subjects responded to NKA inhalation and the mean log<sub>10</sub> PC20 FEV<sub>1</sub> NKA was  $-6.93 \pm 0.15$ . After placebo, the mean log<sub>10</sub> PC20 FEV<sub>1</sub> NKA was  $-6.75 \pm 0.17$  and  $-6.65 \pm 0.11$  at 1.5 h and 24 h post-dose, respectively. The mean "maximal" percentage fall in FEV<sub>1</sub> after inhalation of NKA at which a fall in FEV<sub>1</sub> of  $\geq 20\%$  was reached, was  $-26.6 \pm 1.3$  at screening,  $-27.0 \pm 2.6$  at 1.5 h post-dose and  $-30.5 \pm 2.6$  at 24 h post-dose on the placebo day.

### Effect of SR 48968 on neurokinin A-induced bronchoconstriction

At 1.5 h post-dose, the mean log<sub>10</sub> PC20 FEV<sub>1</sub> NKA was  $-6.25 \pm 0.20$  after SR 48968 and  $-6.75 \pm 0.17$  after placebo ( $p=0.05$ ) (fig. 1). The cumulative dose-response curves of the individual patients are shown in figure 2. Inhalation of NKA caused a dose-dependent bronchoconstriction on both study days; there was a consistent rightward shift in the cumulative dose-response curves after administration

Table 1. – Patient characteristics

Subject No.	Age yrs	Baseline FEV <sub>1</sub> L	Baseline FEV <sub>1</sub> % pred	PC20 methacholine $\text{mg} \cdot \text{mL}^{-1}$
1*	32	4.76	110	5.7
2	34	3.72	88	5.3
3	35	4.84	119	6.9
4	22	4.04	90	6.5
5	36	3.24	73	2.2
6	26	5.04	119	7.0
7*	30	3.16	76	3.3
8	32	3.88	86	8.0
9	33	3.68	91	6.1
10	19	5.28	105	5.5
11	21	4.20	92	2.6
12	24	4.52	92	3.0

\*: patient stopped inhaled glucocorticosteroid treatment 2 weeks before entering the trial. FEV<sub>1</sub>: forced expiratory volume in one second; PC20: provocative concentration causing a 20% fall in FEV<sub>1</sub>.

of SR 48968, with the exception of one case (patient number 1). A protective effect of SR 48968 was noted in the other 11 patients, but a fall of 20% was not reached in three of them. In one of these (patient number 4), PC20 was not reached on either study day. From this patients individual dose-response curve (fig. 2), however, it can be seen that FEV<sub>1</sub> remained unchanged after SR 48968, while it decreased by 10.6% after placebo.

The mean log<sub>10</sub> PC35 sGaw NKA was  $-7.02 \pm 0.28$  after SR 48968 and  $-7.64 \pm 0.19$  after placebo ( $p=0.05$ ) (fig. 1). A protective effect of SR 48968 was noted in 10 out of 12 patients and PC35 was not reached in one of them.

At 24 h post-dose, the mean log<sub>10</sub> PC20 FEV<sub>1</sub> NKA was  $-6.21 \pm 0.17$  after SR 48968 and  $-6.65 \pm 0.11$  after placebo ( $p=0.05$ ) (fig. 1). A protective effect of SR 48968 was observed in eight out of 12 patients and PC20 was not

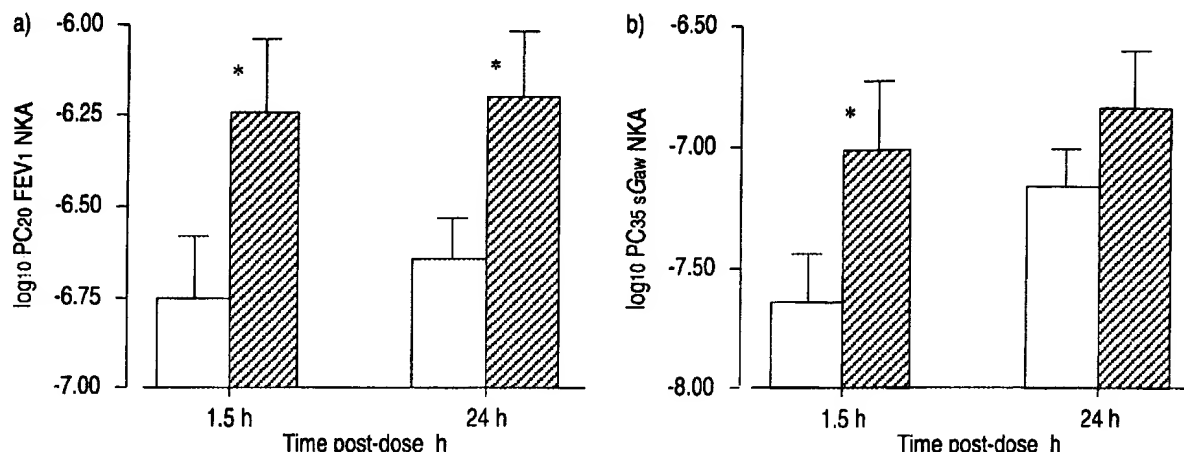


Fig. 1. — Bar graphs representing the mean (SEM) values of a) log<sub>10</sub> of the provocative concentration of neurokinin (NK) A causing a 20% fall in the forced expiratory volume in one second (PC20 FEV<sub>1</sub> NKA) and b) log<sub>10</sub> of the provocative concentration of NKA causing a 35% fall in specific airway conductance (PC35 sGaw NKA) at 1.5 h and at 24 h post-dose, respectively. ▨ : SR 48968; □ : placebo. \*:  $p \leq 0.05$ , statistically significant differences.

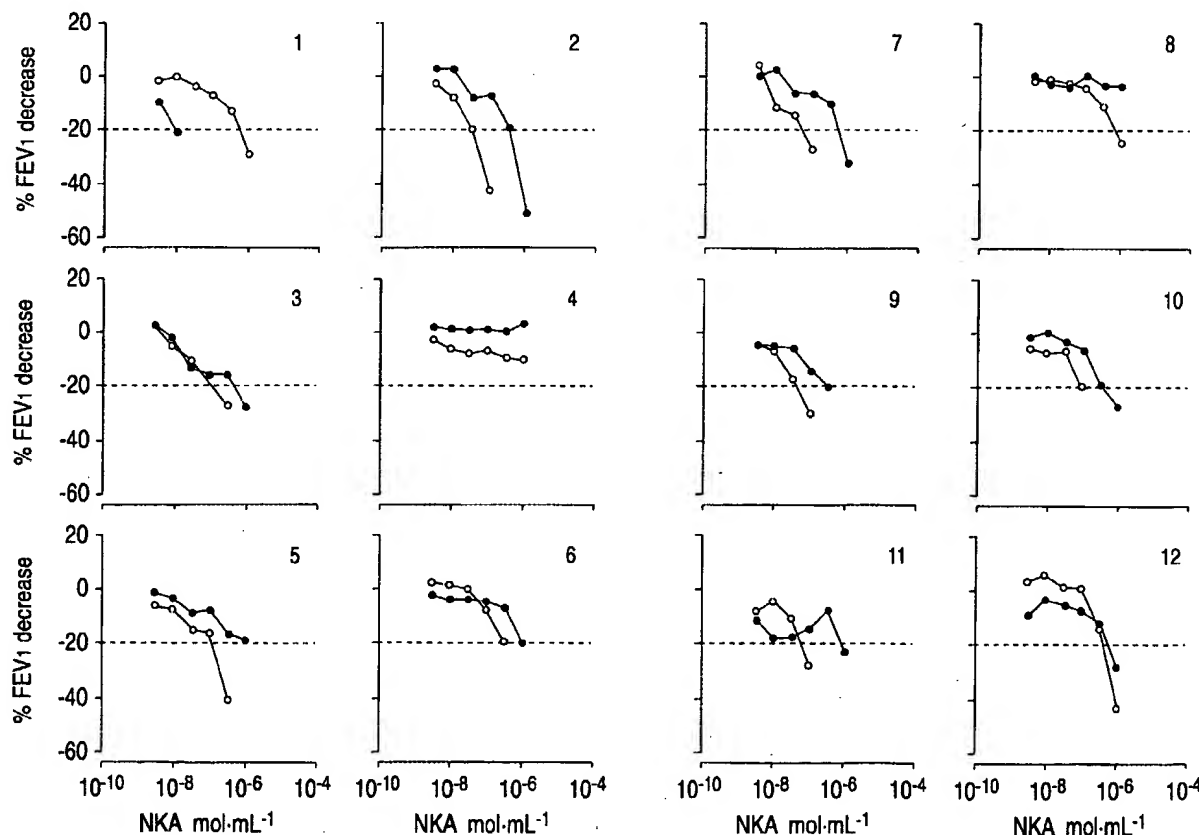


Fig. 2. — Cumulative dose-response curves of individual patients at 1.5 h post-dose. Changes in the forced expiratory volume in one second (FEV<sub>1</sub>) in response to neurokinin A (NKA) inhalation are expressed as the percentage change from the postdiluent baseline FEV<sub>1</sub> value. ● : SR 48968; ○ : placebo. Numbers indicate subject numbers.

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reached in four of them. The mean  $\log_{10}$  PC<sub>35</sub> sGaw NKA was  $-6.85 \pm 0.23$  after SR 48968 and  $-7.17 \pm 0.15$  after placebo (not significant) (fig. 1). A protective effect of SR 48968 was seen in seven out of 12 patients and PC<sub>35</sub> was not reached in two of them.

#### Plasma concentrations of SR 48968

The mean (range) SR 48968 plasma levels at 1.5 h, 3 h and 24 h post-dose were  $38.2$  ( $14.5$ – $92.6$ )  $\text{nmol} \cdot \text{L}^{-1}$ ,  $44.2$  ( $8.0$ – $171.7$ )  $\text{nmol} \cdot \text{L}^{-1}$ , and  $2.8$  ( $0.0$ – $16.3$ )  $\text{nmol} \cdot \text{L}^{-1}$ , respectively. There was no significant correlation between the protective effect and the plasma SR 48968 levels at 1.5 h post-dose, at 24 h post-dose and after pooling of both groups (Spearman's test:  $0.462$ ,  $-0.404$  and  $0.170$ , respectively) (table 2 and fig. 3).

Table 2. – Protective effect\* and plasma SR 48968 levels at 1.5 h and 24 h post-dose

Subject No.	1.5 h		24 h	
	Protection	SR 48968 $\text{nmol} \cdot \text{L}^{-1}$	Protection	SR 48968 $\text{nmol} \cdot \text{L}^{-1}$
1	-1.75	16.123	1.23 <sup>†</sup>	0.000
2	1.02	38.768	0.40	3.261
3	0.52	33.333	-0.06	0.000
4	0.00 <sup>†</sup>	92.572	0.69 <sup>†</sup>	16.304
5	1.45 <sup>†</sup>	47.101	-0.23	1.993
6	0.48	14.493	0.93 <sup>†</sup>	0.000
7	1.00	25.543	0.75	0.000
8	0.69 <sup>†</sup>	44.203	-0.22	2.536
9	0.85	42.754	0.73	3.804
10	0.60	19.022	0.05	2.355
11	1.10	61.594	-0.07	3.261
12	0.14	23.370	1.10 <sup>†</sup>	0.000

\*: the protective effect is defined as the difference between the  $\log_{10}$  of the provocative concentration of neurokinin A (NKA) causing a 20% fall in the forced expiratory volume in one second (PC<sub>20</sub> FEV<sub>1</sub> NKA) value on the SR 48968 day and that on the placebo day. <sup>†</sup>: a 20% fall in FEV<sub>1</sub> was not reached; a PC<sub>20</sub> FEV<sub>1</sub> NKA value of  $3.3 \times 10^{-6} \text{ mol} \cdot \text{mL}^{-1}$  was attributed arbitrarily, thus potentially underestimating the magnitude of the protection.

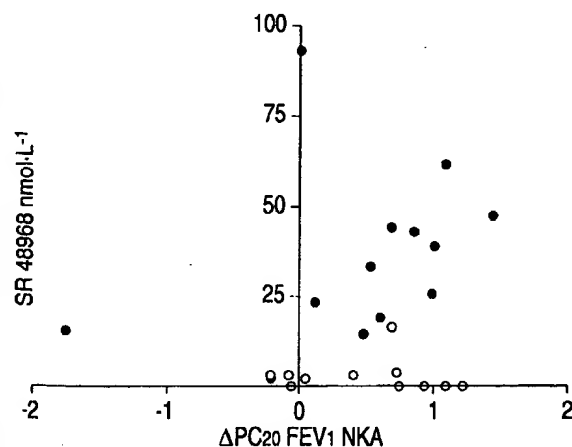


Fig. 3. – Scatterplot of the relationship between the protective effect, expressed as the change in the provocative concentration of neurokinin (NK) A causing a 20% fall in the forced expiratory volume in one second (PC<sub>20</sub> FEV<sub>1</sub> NKA) and the SR 48968 plasma levels. ●: 1.5 h post-dose; ○: 24 h post-dose (see also table 2).

#### Drug safety

SR 48968 was biologically and clinically well tolerated and no serious adverse events were noted. Two non serious adverse events were noted: patients number 7 and 11 both experienced mild frontal headache on the active treatment day; in both cases it subsided spontaneously until complete recovery.

#### Discussion

In this study, it was demonstrated that the tachykinin NK<sub>2</sub> receptor antagonist SR 48968 inhibits NKA-induced bronchoconstriction in patients with asthma. A protective effect was observed both at 1.5 h and 24 h post-dose. The shift in the concentration-response curve, however, was modest and less pronounced than would have been predicted from previous *in vitro* experiments [22]. The calculated mean level of protection against the NKA-induced fall in FEV<sub>1</sub> was 0.5  $\log_{10}$  units at 1.5 h post-dose. However, there were marked interindividual differences in the protection conveyed by SR 48968: complete protection was observed in three patients, whilst a varying degree of partial protection was present in the remaining patients. A PC<sub>20</sub> value of 0.5 log higher than the highest concentration was arbitrarily introduced in the three patients who did not develop a 20% fall in FEV<sub>1</sub> after active treatment. The calculated mean protection is thus very likely to be underestimated. A small protective effect of SR 48968 was still observed 24 h post-dose. Again, the protective effect of SR 48968 was probably underestimated, as an extrapolation of PC<sub>20</sub> FEV<sub>1</sub> NKA was necessary in four subjects.

The results of this study show that at least part of the NKA-induced bronchoconstriction in asthma is mediated via activation of the tachykinin NK<sub>2</sub> receptor. Pharmacokinetic factors such as dose, absorption and penetration may contribute to the apparently limited protection. Poor absorption of SR 48968 from the gastrointestinal tract or poor penetration of SR 48968 from the circulation into the airway mucosa, however, may be excluded. Indeed, the mean plasma level of SR 48968 reached at the moment of the first NKA challenge (1.5 h post-dose) was 38  $\text{nmol} \cdot \text{L}^{-1}$ . In the presence of 30  $\text{nmol} \cdot \text{L}^{-1}$  SR 48968 in an *in vitro* experiment on isolated human airways [22], the rightward shift in the concentration-response curve of [Nle<sup>10</sup>]-NKA (4–10), a specific tachykinin NK<sub>2</sub> receptor agonist, approximated the observed shift in the concentration-response curve for NKA in the present patients. Thus, the serum levels reached in these patients have been shown to be effective *in vitro*. The data therefore strongly suggest that the dose of SR 48968 chosen for this clinical study was adequate. Furthermore, animal data have shown that SR 48968 readily penetrates the airways *in vivo* [26, 30]. In the guinea-pig or the BDE rat *in vivo*, SR 48968 administered intraduodenally (500  $\mu\text{g} \cdot \text{kg}^{-1}$ ) [26] or intravenously (1  $\text{mg} \cdot \text{kg}^{-1}$ ) [30] almost completely abolished the bronchoconstriction caused by NKA or its synthetic analogues.

The finding of residual protection at 24 h post-dose, at a time when virtually all of the circulating active drug had disappeared, suggests a local accumulation of SR 48968 in the airways, the presence of active metabolites of SR



48968 or a long-lasting inhibition of the bronchial tachykinin NK2 receptor. As far as we are aware, no data addressing these issues in humans have been published to date. Following oral administration of 1 mg·kg<sup>-1</sup> SR 48968 to guinea-pigs *in vivo*, there was still demonstrable protection against [Nle<sup>10</sup>]-NKA(4-10)-induced bronchoconstriction at 24 h post-dose; the protection offered was less marked than that found at 2.5 h post-dose [31].

It could be argued that SR 48968 had a nonspecific protective effect. A control bronchoprovocation with a chemically unrelated bronchoconstrictor, such as methacholine, was not included in the study, given the convincing data *in vitro* on isolated human bronchi and *in vivo* in several experimental animal species. Indeed, SR 48968 was shown to be a specific and potent tachykinin NK2 receptor antagonist, as it does not modify concentration-response curves to acetylcholine, histamine, KCl, prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) or specific tachykinin NK1 receptor agonists *in vitro* in human bronchi [22, 25]. In addition, the specificity of SR 48968 for the NK2 receptor has also been confirmed *in vivo* in several animal species, such as rats and guinea-pigs [26, 30, 32].

An important explanation for the modest protective effect of SR 48968 may lie in the fact that NKA is not a specific, but only a preferential tachykinin NK2 receptor agonist; NKA also activates the tachykinin NK1 receptor. Results from pharmacological studies on isolated bronchi from guinea-pigs [33] and humans [25] suggest that the NKA-induced bronchoconstriction is not only due to an NK2 receptor-mediated stimulation of airway smooth muscle, but also to an indirect tachykinin NK1 receptor-mediated activation of airway inflammatory cells such as mast cells, with the release of secondary mediators [34, 35]. In guinea-pig isolated bronchi, the noncholinergic bronchoconstriction produced by electrical field stimulation was shown to be largely mediated by endogenously released tachykinins; pretreatment with specific tachykinin receptor antagonists demonstrated that both tachykinin NK1 and NK2 receptors mediate this contraction [33]. Recently, evidence was provided for the presence of functional tachykinin NK1 receptors in human airways, in addition to tachykinin NK2 receptors. Indeed, NALINE *et al.* [25] reported that stimulation by SP and specific tachykinin NK1 receptor agonists induced a prostanoid-dependent indirect contraction in isolated small human airways. Moreover, upregulation of tachykinin NK1 [19] as well as NK2 [20] receptors has been reported in asthmatic airways.

It is therefore hypothesized that the NKA-induced bronchoconstriction in asthmatics occurs through a combination of direct, tachykinin NK2 receptor-mediated smooth muscle contraction (antagonized by specific tachykinin NK2 receptor antagonists such as SR 48968) and an indirect mechanism involving tachykinin NK1 receptor stimulation (unaffected by SR 48968). The relative importance of NK1 and NK2-mediated bronchoconstrictor responses in asthmatics has not yet been studied.

In conclusion, in this study it was demonstrated that the potent and specific nonpeptide tachykinin NK2 receptor antagonist SR 48968 (saredutant) offers a small (probably underestimated) but significant level of protection against inhaled NKA-induced bronchoconstriction in mild asthmatics. This finding constitutes the first evidence of such inhibition by a tachykinin NK2 receptor antagonist in humans. Studies using tachykinin receptor antagonists in

other settings have been published previously. These involved the low-potency mixed NK1/NK2 receptor antagonist FK-224 and the NK1 antagonists FK-888 and CP-99994. Bradykinin-induced bronchoconstriction in asthmatics was attenuated by 4 mg [36], but not 2 mg [37], of inhaled FK-224. Although this suggested that tachykinin release from airway sensory nerves is involved in responses to bradykinin, this hypothesis could not be confirmed in another study by our group, as no protective effect could be demonstrated of 4 mg of inhaled FK-224 against NKA-induced bronchoconstriction in asthmatics [38]. Inhaled FK-888 was shown to shorten the recovery phase of exercise-induced airway narrowing to some extent, albeit without influencing the maximal fall in sGaw [39]. Finally, intravenously administered CP-99994 did not significantly inhibit hypertonic saline-induced bronchoconstriction or cough in mild asthmatics [40].

Further clinical studies with potent and specific nonpeptide tachykinin receptor antagonists, including neurokinin-1 and combined neurokinin-1/2 antagonists, are clearly needed to explore the therapeutic potential of tachykinin antagonism in asthma [41].

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